NONSTRUCTURAL CARBOHYDRATE CHANGES DURING GROWTH AND STORAGE OF FLORIDA ONIONS (*Allium cepa* L.)

BY

JULIO TOLEDO HEVIA

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TO MY FATHER, TO MY WIFE, AND TO THE MEMORY OF MY MOTHER

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JULIO TOLEDO HEVIA

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Major Department: Horticultural Science

Onions grown at different geographical areas have different storage potential. Cultivars from higher latitudes are known to have more desirable storage characteristics. Available information indicates that changes in the nonstructural carbohydrates during storage are related to the physiology of the bulb. This study comprises an investigation of the changes in the nonstructural carbohydrates during growth and storage of Florida onions.

Carbohydrates accumulated significantly in the bulb during its development. The relative amounts of the individual sugars were similar to those of the stored bulb with the reducing sugars predominating. Fructose and glucose constituted a significantly higher percentage of the total nonstructural carbohydrate fraction than corresponding values found in onions from other geographical areas. The range of degree of polymerization (DP) of the fructans was similar throughout bulbing and storage. Fructans with DP larger than 3 were present in significant amounts only in the bulb. Fructan levels were markedly lower in the leaves with those of DP3 predominating. Fructans with DP larger than 4 were not detected in leaves.

Larger DP fructans predominated and decreased during storage in the outer scales only. The relative distribution of the different DP fructans was different and the total content of these oligosaccharides were significantly lower than those of higher latitude cultivars. Fructans and sucrose increased inwardly whereas fructose and glucose decreased.

The differences in the levels of fructans found in the present experiments from those of high latitude onions together with the results of the storage trials indicated a possible role for these carbohydrates in the storability of the bulbs. Higher fructan-containing bulbs appeared to have better storability.

CHAPTER I
INTRODUCTION

The ability of fresh products to withstand storage is important because it allows for a more even distribution of the commodities throughout the year. Good storage onions (*Allium cepa* L.) are related to high dry matter content together with long photoperiods and dry weather conditions during bulb maturation. For these reasons, in the United States, production of good keeping cultivars is limited to the northern States.

In Florida, production of spring onions has potential for success because of the likelihood of obtaining high yields. The nearness to eastern markets appears to favor the marketing of the onions produced. However, the possibility of rainfall and high air humidity makes it difficult to field dry (cure) the bulbs. Since the bulbs must be shipped immediately following harvest, over-supply of spring onions can create serious marketing problems. Low latitude onions are known not to be good keepers and this is thought to be, in part, related to their relatively low solids.

Methods of extending the storage period of the bulbs might be possible if the biochemistry involved in the physiology of the stored bulb were better understood . Growth regulator changes have been the subject of much work but no clear picture of the biochemical control mechanism has yet emerged.

An alternative approach is to measure changes in carbohydrates during storage since they have been found to be closely linked to onion bulb physiology during storage (Kato, 1966). The major nonstructural carbohydrates in onions include fructose, glucose,and sucrose together with a series of oligosaccharides, the fructans. These carbohydrates may account for over 80% of the dry weight of the bulbs.

Changes in the overall content of carbohydrates during development and storage of onions have been reported primarily for high latitude onions. Reporting carbohydrates as reducing or nonreducing sugars or total soluble solids gives little indication of the qualitative and quantitative changes of the individual sugars present in the bulb. These have not been studied in detail for low latitude onions.

The goal of this research was to study the quantitative and qualitative changes in nonstructural carbohydrates in Florida onions. The changes in nonstructural carbohydrates in the onion plant during bulbing are presented in Chapter III. Chapter IV describes the changes in fructans in the stored bulb and changes in fructose, glucose, and sucrose are presented in Chapter V. The results are discussed in terms of the possible role of these sugars in the storability of the bulbs.

CHAPTER II
LITERATURE REVIEW

Fructans

Introduction

The study of the naturally occurring polymers of fructose (fructans) began in 1805 with the discovery of inulin in the rhizomes of Inula helenium (Archbold, 1940). There are 2 main types of fructans: the inulin type, in which the fructofuranose residues are linked together by β-2:1 glycosidic linkages; and the phlein (levan) type, in which the fructofuranose are linked by β-2:6 glycosidic linkages (Goodwin and Mercer, 1983). Both types carry a D-glucosyl residue at the end of the chain, linked as in sucrose. A third type, the branched type fructans, may have several short branches consisting usually of a single fructose residue. These can have an inulin or phlein backbone (Meier and Reid, 1982).

Fructans are soluble in water but not in alcohol (Archbold, 1940). These carbohydrates are usually isolated by exhaustively pre-extracting the tissues with 90 to 95% ethanol to remove the simple sugars (Archbold, 1940; Archbold and Barter, 1935; Chen and Hou, 1981;

Grotelueschen and Smith, 1968; Holligan et al., 1973; Khodzhaeva and Ismailov, 1979; Smith, 1967, 1968; Smith and Grotelueschen, 1966; Volenec and Nelson, 1984a,b). The bulk of the fructans are then extracted either with lower concentration of aqueous ethanol or with water.

The separation and identification of fructans is achieved by a variety of chromatographic procedures (Kandler and Hopf, 1982). The most common are paper chromatography, thin-layer chromatography, column chromatography and electrophoresis. These methods have been extensively used in the study of onion fructans (Bacon, 1957, 1959b; Bose and Srivastava, 1961; Darbyshire, 1978; Darbyshire and Allaway, 1981; Darbyshire and Henry, 1978, 1979, 1981; Henry and Darbyshire, 1978, 1979, 1980; Lolas and Markaris, 1973; Pant et al., 1962; Rutherford and Whittle, 1982; Singh and Bhatia, 1972; Sinha and Sanyal, 1959; Srinivasan et al., 1953).

Fructans are susceptible to enzymic and acid destruction (Kandler and Hopf, 1982). When the plant material cannot be extracted immediately after harvest it should be stored in a deep freeze (-20°C) or freeze-dried. Storage in a refrigerator (0°C) does not prevent the autolytic breakdown processes of these carbohydrates. Fructans are not colored by iodine and most of them are attacked to a greater or lesser extent by yeast invertase, although the attack is rather slow (Archbold, 1940).

Fructans of higher plants are little understood physiologically, even though they are widespread in occurrence and can reach very high levels in particular tissues. Fructans are deposited in large quantities (up to 80% dry weight) in storage organs (tubers, bulbs, rhizomes), and, to a much lesser extent, also in seeds (Halmer and Bewley, 1982; Hirst, 1957; Meier and Reid, 1982; Pollock and Ruggles, 1976). The role of fructans as reserve carbohydrates has been reported elsewhere (Halmer and Bewley, 1982; Hirst, 1957; Meier and Reid, 1982; Pollock and Ruggles, 1976). The possibility of these carbohydrates being associated with cold resistance in storage organs (Edelman and Jefford, 1968) and in grasses (Eagles, 1967; Pollock et al., 1979) seems to be related to the ability of fructans to act as osmoregulators. Fructans are thought to accumulate in the vacuoles as solutes or, at least, in a highly hydrated state which makes them osmotically active (Edelman and Jefford, 1968; Frehner et al., 1983; Kandler and Hopf, 1982; Wagner et al., 1983; Wagner and Wiemken, 1984). Furthermore, the mean molecular size of fructans can be easily changed using only a simple transferase system (Wagner et al., 1983).

Occurrence in Higher Plants

In angiosperms fructans are present in the more highly evolved families of both dicotyledons and monocotyledons. It has been shown that species from the following families of the dicotyledons contain fructans: Asclepiadaceae, Boraginaceae, Campanulaceae, Compositae, Gentianaceae, Goodeniaceae, Lobeliaceae, Malpighiaceae, Primulaceae, Stylidiaceae, and Violaceae (Meier and Reid, 1982). These carbohydrates have been most extensively studied in the Compositae (Chandorkar and Collins, 1972, 1974; Collins and Chandorkar, 1973; Deacon and Rutherford, 1972; Edelman and Jefford, 1968; Flood et al., 1970; Perchorowicz et al., 1984) where a number of species have been found to store fructans as a reserve material, especially in the rhizomes and roots.

In monocotyledons, fructans have been found to occur in members of the Lilliaceae, Amaryllidaceae, Iridaceae, and Poaceae. Wille (1917) found that the Poaceae store starch, sucrose, fructans and hemicelluloses in their vegetative tissues. Bender and Smith (1973) found no correlation between starch and fructan storage in C_4 and C_3 species. In the Amaryllidaceae fructans occur along with starch in Lycoris sp. and Narcissus ap. (Meier and Reid, 1982). According to these authors, in the Lilliaceae fructans are present as the main reserves in rhizomes, tubers and bulbs, along with starch.

Only inulins are known to occur in the dicotyledons whereas all types of fructans have been found in monocotyledons (Meier and Reid, 1982). Phlein type fructans have been isolated mainly from temperate grasses and their degree of polymerization (DP) varies from about 10 to 260 (Grotelueschen and Smith, 1968; Pollock et al., 1979; Tomasic et al., 1978). Inulins occur rarely in monocotyledons but they have been found in garlic (Allium sativum) by Das and Das (1978) as well as in the rhizomes of the grass Polygonatum odoratum by Tomoda et al. (1973). Branched fructans have been reported in a number of monocotyledons (Arni and Percival, 1951; Medcalf and Cheung, 1971; Montgomery and Smith, 1956, 1957; Srinivasan and Bhatia, 1953).

Synthesis in Higher Plants

Fructan synthesis has been extensively studied in Helianthus tuberosus L. by Edelman and his coworkers, whose findings were compiled in a review paper by Edelman and Jefford (1968). Fructan synthesis is by a series of fructosyl transferases with sucrose as the primary donor (Edelman and Jefford, 1968).

In tubers of Jerusalem artichoke, the enzyme sucrose:sucrose fructosyl transferase (SST) converts 2 sucrose molecules to a trisaccharide and glucose, (Edelman and Dickerson, 1966; Perchorowicz et al., 1984). This enzyme is not an invertase and it is unable to promote polymerization above the trisaccharide level. An enzyme with activity similar to that of SST in Jerusalem artichoke has been found in other dicotyledons (Singh and Bhatia, 1971) and also in monocotyledons (Bhatia and Nandra, 1979; Satyanarayana, 1976a,b,c), including onions (Scott et al., 1966; Henry and Darbyshire, 1980).

Chain elongation is due to the enzyme fructan:fructan fructosyl transferase (FFT) which catalyzes the transfer of single fructose residues to the growing fructan polymer (Edelman and Jefford, 1968). Henry and Darbyshire (1980) found FFT activity in onions. According to the hypothesis put forward by Edelman and Jefford (1968), SST is located in the cytoplasm whereas FFT is thought to be situated at the tonoplast. Since the activities of SST and FFT could not be separated from each other in the vacuoles of the mesophyll cells of barley (Hordeum vulgare), the possibility that only one enzyme is involved in the synthesis of fructans in leaf blades of this species was recently proposed (Wagner et al., 1983; Wagner and Wiemken, 1984).

Chandorkar and Collins (1974) suggested the possibility that fructosyl transferases are inducible enzymes, since leaf discs of certain composites which do not normally accumulate fructans in their leaves can be induced to do so by incubation with fructose, glucose or sucrose. This view was expressed also by Pontis (1970). Fructans can also be induced to accumulate in leaf blades of barley by impeding the export of photosynthates (Wagner et al., 1983; Wagner and Wiemken, 1984).

The metabolism of the phlein type fructans in the leaves of temperate grasses differs substantially from that of the members of the Compositae and Alliaceae (Pollock, 1982b). Synthesis in grasses can occur in the absence of detectable quantities of the trisaccharide intermediates found in other species, but there are reports of the occurence of trisaccharides in some grasses (Bacon, 1959b). Low temperatures (5-8°C) have been found to enhance the synthesis of fructans in grasses (Del Campillo and Pontis, 1984; Eagles, 1967; Nelson and Coutts, 1984; Pollock, 1979, 1981; Pollock and Ruggles, 1976; Smith, 1968; Smith, 1967; Wagner and Wiemken, 1984). Fructan synthesis in the leaves of some monocotyledons has also been found to be stimulated by fungal infection (Chen and Hou, 1981; Holligan et al., 1973).

Degradation in Higher Plants

Depolymerization of fructans in tubers of Jerusalem artichoke (*Helianthus tuberosus*) is due to the presence of 2 exohydrolases, termed A and B by Edelman and Jefford (1964). Both enzymes, proposed to be at the tonoplast, are inhibited by sucrose which thus provides a negative feedback regulation (Edelman and Jefford, 1968). Rutherford and Deacon (1972a,b) isolated 2 hydrolases from dandelion (*Taraxacum officinale*) which showed similar characteristics to those from Jerusalem artichoke, but they were not inhibited by sucrose.

Smith (1976) demonstrated the presence of an exohydrolase in the culm base tissue of tall fescue (Festuca arundinacea). The enzyme was specific for phlein fructans and did not attack inulin.

Onions

Bacon (1957, 1959b, 1960) found a series of non-reducing oligosaccharides in onion bulbs. These oligosaccharides were converted to a mixture of glucose and fructose by the action of yeast invertase, an indication that they consist chiefly of β-fructofuranose residues. Two major trisaccharides were isolated from water extracts of the bulbs (Bacon and Bell, 1953; Lemieux et al., 1956). The trisaccharides were identified as 1^F-fructosylsucrose (isokestose) and 6^G-fructosylsucrose (neokestose).

Fructans are distributed unevenly in the mature onion bulb, being almost absent from the outer scales, whereas they may constitute more than half of the soluble carbohydrate in the inner ones (Bacon, 1957, 1959b). There is more oligosaccharide at the base of the scales than at their tops (Bacon, 1957, 1959b). These authors found no evidence for the presence of fructose polysaccharides like those found in many other monocotyledons. Their paper chromatograms indicated a DP of up to 8. Bose and Srivastava (1961) confirmed the findings of the earlier workers. Singh and Bhatia (1972) were able to identify fructans of up to DP7 in their paper chromatograms from onion bulb juice whereas Srinivasan et al. (1953) reported oligosaccharides of DP6. Lolas and Markaris (1973) suggested the presence of nine fructans in onions, 2 trisaccharides, 3 tetrasacharides, 2 pentasaccharides and 2 unidentified higher polymers.

Working with only one onion bulb Darbyshire and Henry (1978) identified fructans of up to DP11 in 80% ethanol extracts using gel permeation chromatography (Bio-Gel P-2) eluted with water. Confirmation of the nature of the fructans separated was achieved by hydrolysis of the individual fructans and determination of the fructose:glucose ratio. Their results showed the presence of a single glucose unit per fructan. Fructans increased in concentration in the inner scales. They also found that the concentration of each fructan declined with increasing molecular weight and this relationship was consistent for all the scales. As a percentage of total carbohydrate, there was a decline in total fructan from 51% in the innermost scale (youngest one) to 21% in base 1 (oldest one) whereas fructose levels increased centrifugally within the bulb from 7 to 31%. The authors interpreted this change to be due to a hydrolysis of fructan to free fructose which achieves osmotic adjustment while the scales expand during bulbing.

Using Bio-Gel P-2, Darbyshire and Henry (1979) found that onion cultivars with a higher percentage dry weight contained a higher concentration of greater DP fructans. They also found that as the percentage dry weight of the different cultivars increased, the total fructan content also increased.

Fructan and Other Carbohydrate Changes During Plant Development

Onions

The literature in this field is sparse and no specific studies on the changes in fructans of onions during their development have been reported. Butt (1968) found total sugars accumulated in onion bulbs as they developed. Lercari (1982b) found that reducing sugars accumulated both in the leaf blades and in the bulbs whereas there was accumulation of oligosaccharides only in the bulb.

Steer and Darbyshire (1979) studied the carbohydrate content of leaves of bulbing onions. The only fructan quantified was the trisaccharide which was found to increase towards the leaf base. Darbyshire et al. (1979) followed the diurnal variations in nonstructural carbohydrates of juvenile onion leaves. They found trace amounts of trisaccharide occasionally but they were not able to quantify that carbohydrate fraction. These authors estimated fructans in the juvenile onion plants from differences between the total carbohydrate in ethanol extracts and individual analysis of fructose, glucose and sucrose using gas chromatography. They found that fructans may account for 7% to 15% of the carbohydrate content in the base of the leaves whereas only trace amounts were estimated for the upper part of the leaves. They concluded that fructans in juvenile plants are present in small amounts. They suggested using more mature plants, perhaps shortly after bulbing, better to characterize fructan accumulation.

Henry and Darbyshire (1979) studied the distribution of fructan metabolizing enzymes in 30 bulbing onion plants. Their results indicated that most fructan synthesis occurs in the inner leaf bases and most fructan hydrolysis occurs in the outer leaf bases of the onion bulb. It has been suggested that fructans may be hydrolysed to provide osmotic adjustment as the leaf base cells expand during bulbing (Darbyshire and Henry, 1978). Steer (1982) found no differences in the fructan levels of mature onion bulbs from plants grown at different temperature regimes. Nilsson (1980) found that the amount of free fructose increased during the maturation period of the bulbs prior to harvest. He interpreted this as due to hydrolysis of fructans.

Other Fructan-Containing Species

In tubers of Jerusalem artichoke seasonal changes of fructans and other carbohydrates were studied by Bacon and Loxley (1952) and Dickerson and Edelman (1966). The tubers of this species are the organs of vegetative propagation, and each tuber has a life span of about 1 year. After the initiation of a tuber, there is a period of rapid growth in the late summer with a massive increase in fructans. According to these authors, the formation of fructans appears to be in response to a change in sucrose translocation from upwards to downwards.

When tubers sprout, the polymerized fructose is largely consumed in supporting the growth of the new plant (Jefford and Edelman, 1961). They observed a sharp decrease in the dry weight of the tubers during the first weeks of growth of the new shoots. At least 80% of this loss was accounted for by disappearance of fructans. Changes in the fructan content of mother tubers stopped when the daughter plants were removed, indicating that a controlling influence in the normally sprouting tuber is, in fact, the daughter plant. Rutherford and Flood (1971) demonstrated seasonal changes in hydrolase and invertase activities in the tubers of Jerusalem artichoke.

In roots of dandelion the fructan reserves are depleted in spring when flowering starts, and during late summer the reserves are restored. The hydrolase activity is highest in late spring (Rutherford and Deacon, 1974).

In chicory (Cichorium intybus) fructans accumulate in early summer (Bhatia et al., 1974). According to Chubey and Dorell (1977) the fructan content in this species is highest during the fall. When chicory is forced, oligofructans in the central axis decrease by 60% while the reducing sugars increased (Rutherford and Phillips, 1975).

In grasses, the fructan content varies considerably during development. Fructans in the stem bases of both Phleum pratense and Bromus inermis decreased in spring and increased again in summer (Smith, 1967). Differences in the changes in DP of fructans were observed throughout the growing season for these 2 species.

Barnell (1938) noted that the fructans in the ears of wheat are transitory reserves which disappear during maturation of the seeds in which large amounts of starch are formed. Escalada and Moss (1976) also found the fructan content in wheat kernels reached a maximum before the phase of rapid starch synthesis. Blacklow et al. (1984) have recently found that fructans in the internodes of winter wheat polymerized and depolymerized as grain filling progressed. Gel permeation was used to separate the polymers of fructose and there was little indication that the maximum DP was greater than 9. Fructans of DP greater than 5 increased rapidly to a maximum of 27% of the nonstructural carbohydrate fraction at 24 days after anthesis while those of DP3 and 4 reached a maximum concentration of 9% about 10 days after anthesis.

Polymerization and depolymerization of the fructans were explained by the authors in terms of a changing balance between net photosynthesis and rates of grain growth.

Judel and Mengel (1982) studied the content of nonstructural carbohydrates in leaves and culms of spring wheat (*Triticum aestivum* L.) during the grain filling period. Fructans accumulated to a maximum in leaves and culms during the first 2 to 3 weeks after anthesis. By this time, the amount of fructans stored in leaves and culms was more than twice as much as the quantity of stored starch. The authors concluded that fructans play a major role as storage carbohydrates during the grain filling period in spring wheat.

In *Phleum pratense* fructan accumulation was maximal at low temperatures and under long days, presumably because this was when the excess of photosynthate supply over demand was greatest (Pollock 1981).

Labhart et al. (1983) found that an apparent threshold of nonstructural carbohydrates was neccesary for synthesis of fructans with DP greater than 20 in meadow fescue (*Festuca pratensis* Huds). This concentration was near 12% of the dry weight for leaf tissue, about 6% for stem base tissue and 2.5% for root tissue. The DP range of the high DP fructan group was 43 to 50 for leaf tissue, 31 to 93 for stem base tissue, and 27 to 31 for roots. These characteristics seemed to be mostly tissue dependent with less effect from temperature and radiation.

Concentration of fructans, the major water-soluble carbohydrates in tall fescue (Festuca arundinacea Scherb.), was highest in leaf intercalary meristem, and decreased rapidly with age (Volenec and Nelson, 1984a). The high concentration of storage carbohydrate in actively growing tissue was not expected although Jones and Nelson (1979) found high concentrations (about 50% dry weight) of water-soluble carbohydrates in terminal meristem tissues of tall fescue. About 70% of the water-soluble carbohydrate fraction of tall fescue meristem tissue was fructan. Significant quantities of fructans have also been reported within bases of elongating leaves of other grasses (Kemp and Blacklow, 1980; Munns et al., 1982, Munns and Weir, 1981; Volenec and Nelson, 1984b). The function of fructans in intercalary meristems is unknown. However, Volenec and Nelson (1984) speculate that they might serve as a pool of carbon that is utilized when supply of carbohydrate from photosynthesis cannot meet demand or, alternatively, they may have a role in osmotic regulation within the meristem.

Fructan and Other Carbohydrates Changes During Storage

Onions

Sugar content of onion bulbs has been measured in several studies. Bennett (1941), measured reducing sugars (as glucose) and non-reducing sugars (as sucrose) of

'Ebenezer' onions prior to storage and after 3.5 months of storage. In the first year the bulbs contained, on a dry weight basis at the beginning of storage, 60% non-reducing sugars and 5.2% reducing sugars. In the second year the contents were 42% and 17%, respectively. The percentage loss of total sugars varied from 3% to 8%. Reducing sugars (glucose) increased during storage at low temperatures (1-3°C), where during the first year they rose from 5% to 21% and, during the second year, from 17% to 29%. Karmarkar and Joshi (1941) and Yamaguchi et al. (1957) also found that reducing sugars increased during low-temperature storage. Hurst et al. (1985) have recently studied the changes in total sugars during storage in low latitude cultivars. They found the level of sugars to increase at 4°C storage whereas it did not change significantly after 4 weeks at 1°C.

Misra and Panda (1979) measured total soluble solids using a hand refractometer and found them to decrease during storage whereas the opposite was found by Musa et al. (1974).

Darbyshire (1978) and Rutherford and Whittle (1982) are the only papers available on the changes of fructans in stored onions. Using gas chromatography the only fructan detected by Darbyshire (1978) in the 75% ethanol fraction was the trisaccharide. In a comparison of storage temperatures (4°C, 15°C, 25°C, 37°C) he found fructose levels increased rapidly after 8 weeks in bulbs stored at $4^{o}C$. This was attributed to low-temperature hydrolysis of fructans. Sucrose concentrations increased from the outer scales to the innermost ones. Glucose and fructose also tended to increase centripetally within the bulb.

Rutherford and Whittle (1982) measured the carbohydrate changes in the onion cultivar 'Robusta' stored at $4^{o}C$. Individual carbohydrates were determined in the 80% ethanol extract. Simple sugars and trisaccharide were measured using gas chromatography. Other fructans were assessed using descending paper chromatography. They found mainly DP3 and DP4 fructans with very small quantities of DP5 and DP6 and negligible, if any, amounts of fructans with higher DP. The main change observed in the stored bulbs was the hydrolysis of fructans to reducing sugars (Rutherford and Whittle, 1982). The amount of fructose and the weight of the innermost scales in freshly harvested bulbs correlated with the length of time the onions could be held in storage (Rutherford and Whittle, 1982). In another study, the activity of alkaline invertase present in the stored bulbs was directly related to their storage longevity (Rutherford and Whittle, 1984).

Other Fructan-Containing Species.

In Jerusalem artichoke a partial depolymerization of inulin occurs during cold storage in winter, whereas complete depolymerization to sucrose and fructose takes place during sprouting (Edelman and Jefford, 1968).

Jefford and Edelman (1963) found that higher DP fructans were converted to shorter-chain ones in dormant tubers of Jerusalem artichoke and that there was an associated increase in the combined glucose content. Storage at 2°C, both accelerated and greatly magnified this process; storage at 20°C considerably depressed it (Jefford and Edelman, 1963). They also found this change not to be controlled by the dormant buds. Jefford and Edelman (1963) likened this depolymerizing effect of low temperature with the sweetening of potatoes which occurs at temperatures below 5°C and which is known to be due to production of sugar from starch (Arreguin-Lozano and Bonner, 1949; Porter and Rees, 1954; Nowak, 1977; Bailey et al., 1978).

Rutherford and Weston (1968) studied the effect of cold storage (3°C) on the carbohydrates in Jerusalem artichoke tubers in comparison with roots of chicory and dandelion which have similar carbohydrate composition. They found cold storage of chicory and dandelion roots caused breakdown of inulin to lower DP fructans. These changes were nearly complete within the first 6 weeks of cold storage. Storage of chicory roots at higher temperatures decreased the rate of sugar breakdown. Cold storage caused less breakdown of inulin in Jerusalem artichoke tubers. The general pattern of fructan breakdown found by Rutherford and Weston (1968) in all 3 roots and tubers was similar to that reported previosly in artichoke (Bacon and Loxley, 1952; Jefford and Edelman, 1961, 1963) and also similar to the changes in starch content found in potato during storage. The enzymes involved in fructan breakdown during cold storage have been studied by several authors (Deacon and Rutherford, 1972; Flood et al., 1967, 1970; Rutherford and Deacon, 1972a,b; Rutherford et al., 1969; Singh and Bhatia, 1971).

Paper chromatographic analysis of carbohydrates from tulip bulbs revealed a substantial increase in the content of sucrose and short-DP fructans during the first 2-4 weeks of storage at $5^{o}C$ (Moe and Wickstrom, 1973). After 12 weeks at $5^{o}C$, the content of oligosaccharides decreased. The initial increase in fructan content was accompanied by a corresponding starch decrease. High temperature storage ($21^{o}C$) led to comparatively slight changes in the sucrose and fructan content of the bulbs. The concentration of fructose and glucose showed slight and irregular variations in bulbs kept at either temperature. Similar results to those of Moe and Wickstrom (1973) were found by Haaland and Wickstrom (1975).

Davies and Kempton (1975) found that starch content decreased in tulip bulbs stored at $9^{o}C$ whereas it increased during storage at $18^{o}C$. Cooled bulbs contained considerably more sucrose than uncooled bulbs both at the end of storage and during the first 8 weeks of growth. Free fructose was extremely low at all times. Glucose accumulated considerably in the tissues once the shoots began to grow rapidly.

CHAPTER III
CARBOHYDRATE METABOLISM IN RELATION TO BULBING

Introduction

Bulb development in the onion plant begins in response to the stimulus of long days (Garner and Allard, 1923; Magruder and Allard, 1937; McClelland, 1928). Bulb formation is accompanied by carbohydrate accumulation (Butt, 1968; Kato, 1965; Wilson, 1934). The nonstructural carbohydrates in onions include mainly fructose, glucose and sucrose together with a series of oligofructosides, the fructans (Bacon, 1957, 1959b; Darbyshire and Henry, 1978).

Relatively little is known about the carbohydrate metabolism of the onion plant during its development and most of the available data refer to high latitude cultivars (Butt, 1968; Darbyshire et al., 1979; Lercari, 1982a,b; Steer, 1980, 1982; Steer and Darbyshire, 1979).

The present work reports on the carbohydrate metabolism of a low latitude onion cultivar in relation to the bulbing process. The data are intended to complement studies done on the carbohydrate metabolism of the stored bulbs.

Materials and Methods

Plant Material

Onions (Allium cepa L.) of the yellow cultivar 'Granex 429' were grown at the Hastings Agricultural Research and Education Center.

Sampling for Carbohydrate Analysis

Sample bulbs were taken every 15 days starting on March 10, 1984 (103 days after transplanting) and until 1 week before harvest. Rapid bulb development started 133 days after transplanting. Five samplings were done for bulbs whereas only 4 were possible for leaves since these were completely dry at the last sampling period. Twenty-five bulbs constituted a sample. Whole plants were placed into plastic bags and transfered from the field to the laboratory in an iced box. Samples were always taken at about the same time of the day (9am) to minimize any variation due to diurnal fluctuation in the carbohydrate levels (Darbyshire et al., 1979). The bulbing ratio (bulb diameter/neck diameter) of each plant was determined at each sampling date to indicate the degree of development of the bulb. The plants were divided into leaves and bulbs and these were further divided into sections: upper (upper half); lower (lower half); outer (3 outermost scales, 2 outermost fully developed leaves); and inner (3 innermost scales, 2 innermost developing leaves). The samples were stored at -40°C until used for sugar analysis. The dry weight of the individual sections was determined by drying additional samples to a constant weight (65°C for 72 hours). The number of scales per bulb ranged from 8 to 10. Each plant had between 6 and 8 leaves.

Preparation of Ethanol-Insoluble Powders

A 15 g sample of tissue was homogenized in 90% ethanol for 1.5 minutes using a Sorvall homogenizer. After boiling for 25 minutes to inactivate endogenous enzymes, the homogenate was stored at -40°C overnight to enhance the precipitation of the alcohol-insoluble solids. The homogenate was filtered through Miracloth (Calbiochem-Behring Corp.) and the ethanol-insoluble powder (EIP) washed twice with 100 ml of 100% ethanol. The dried EIP was analyzed for fructans and the ethanol-soluble fraction was used for analysis of fructose, glucose and sucrose

Fructan Extraction and Analysis

Ethanol-insoluble powder (150 mg) was placed in 10 ml of Na-acetate buffer (25 mM, pH 5) and stirred for 15 minutes in a hot water bath (80°C). The mixture was filtered through glass microfibre filter discs (Whatman) and the filtrate concentrated to yield a total sugar concentration of about 1 mg ml^{-1}. Total sugar was determined by the phenol-sulfuric method (Dubois et al., 1956). Approximately 1.5 ml (about 1.5 mg) were applied to a water-jacketed Bio-Gel P-2 column (46 cm x 1.5 cm; -400 mesh) operated at 45°C and eluted with Na-acetate buffer

(25 mM, pH5) containing 100 mM NaCl at a flow rate of 0.2 ml $\min^{-1}$. One-ml fractions were collected in a 2070 Ultrorac II fraction collector (LKB Bromma), and 0.5 ml of these were analyzed for total fructose using the resorcinol assay (McRary and Slattery, 1945). The elution position of the sugars, glucose, sucrose, raffinose, stachyose and blue dextran were used as standards. Quantitative determinations of the total fructans were done by directly analyzing aliquots (0.5 ml) of the filtrate.

Analysis of Ethanol-Soluble Sugars

Individual sugars were determined by the analysis of their trimethylsilyl derivatives as described by Styer (1982). Aliquots (0.5 ml) of the ethanol extract were dried in a heating block set at 55^{o}C. Oximes of the dried sugars were made by addition of 0.1 ml of STOX Oxime-Internal Standard Reagent (Pierce Chemical Co.) containing 6 mg phenyl-β-D-glucopyranoside/ml pyridine as an internal standard. After incubation for 30 minutes at 70^{o}C the vials were cooled to room temperature before silylation. The oxime-sugars were silylated with 0.1 ml of TSIM trimethyl silylimidazole reagent (Pierce Chemical Co.) followed by vortexing the sample 15 seconds. The samples were incubated for 30 minutes at room temperature before chromatographic analysis. One microliter of the silylated sample was injected into a Hewlett Packard 5710A gas chromatograph fitted with a hydrogen flame ionization detector. The sugars were resolved on a glass column (0.75

cm, 1.8m) packed with 3% OV-17 on 80/100 mesh Chromosorb W-HP (Supelco).

An initial temperature of 180^{o}C was maintained for 2 minutes followed by a rise in temperature at a rate of 8^{o}C/min to 270^{o}C and holding for 2 minutes. Injection port temperature was 300^{o}C. The carrier gas was helium. Sugars were expressed as mg g^{-1} dry weight. A mixture of fructose, glucose and sucrose, each at a concentration of 1 mg ml^{-1}, was used as a standard for the identification and quantification of the sugars in the chromatograms.

Starch Determination

Samples of EIP (20 mg) from bulbs and leaves were assayed by amylase digestion. Amylase (135 units) was added to a 10 ml of Na-phosphate buffer (20 mM, ph 6.8) containing 0.02% Na-azide and 3mM calcium chloride. One ml of soluble starch (1 mg ml^{-1}) was used as control. Boiled control treatments were also included. One ml of the amylase-containing solution was added to the EIP samples and the reaction mixtures were made up to 2 ml with Na-phosphate buffer. Amylase digestion was carried out overnight in a water bath at 34^{o}C with constant agitation. After the reaction period, the samples were filtered through glass microfibre filter discs (Whatman). The supernatant (0.5 ml) was analyzed for reducing sugars (Somogyi, 1952).

Results

Starch levels were negligible in both bulbs and leaf blades as determined by the amylase reaction. Changes in the relative amounts of the different degree of polymerization (DP) fructans in the bulbs and leaves were determined for each sampling date. Typical qualitative results are presented in Fig. 3-1. Additional sampling dates not shown in the figures showed similar trends. Fructan composition of the inner scales was similar in both the upper and lower portion of the bulb where the larger DP chains predominated (Fig. 3-1). Fructan composition of the outer scales differed somewhat in the upper and lower bulb, the latter having a relatively higher amount of larger DP oligomers. Only members of the short DP fructans, mainly tri- and some tetrasaccharides were predominant in the leaf blades (Fig. 3-2). A decreasing trend in the relative amounts of fructans was apparent from the upper to the lower part of the leaf blades.

Sampling date (stage of development) had a significant effect on the distribution of all the carbohydrate fractions in bulbs and leaves (Table A-1) and the results are presented in Fig. 3-3, A-E. Changes in bulbing ratio over time (Fig. 3-3, F) are presented to relate the observed changes in carbohydrates to the developmental stages of the bulb. After an initial decrease, the level of fructans in the bulbs (A) increased between bulbing

Fig. 3-1. Bio-Gel P-2 chromatography of fructans from onion bulbs of the cultivar 'Granex 429' at different stages of development (days after transplanting). Elution fractions for glucose, sucrose, stachyose, and blue dextran are marked by I-V, respectively.

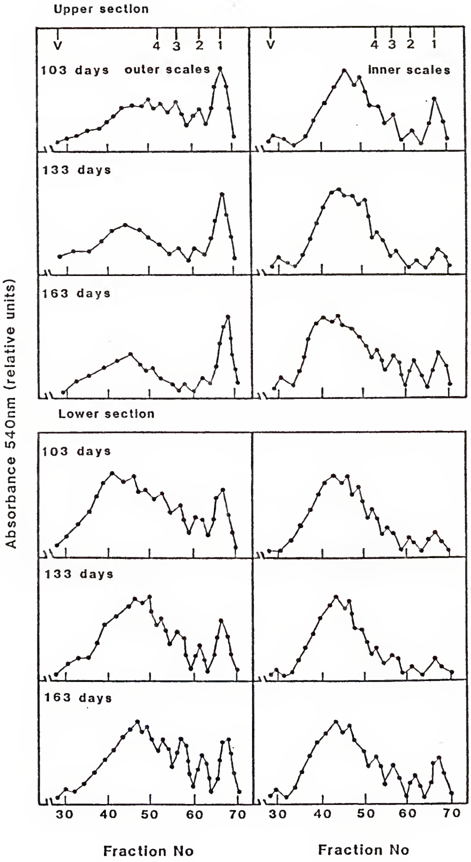

Upper section
V
4 3 2 1
103 days
outer scales
inner scales
133 days
163 days
Lower section
103 days
133 days
163 days
Absorbance 540nm (relative units)
30
40
50
60
70
Fraction No

Fig. 3-2. Bio-Gel P-2 chromatography of fructans from onion leaves of the cultivar 'Granex 429' at different stages of development (days after transplanting). Elution fractions for glucose, sucrose, stachyose, raffinose, and blue dextran are marked by 1-V, respectively.

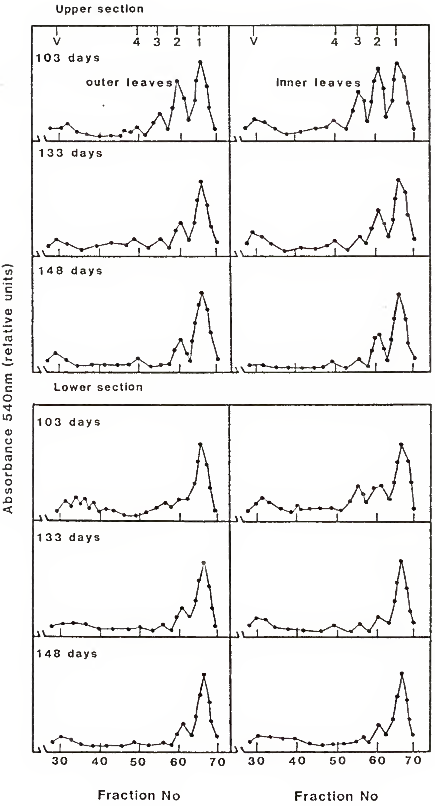
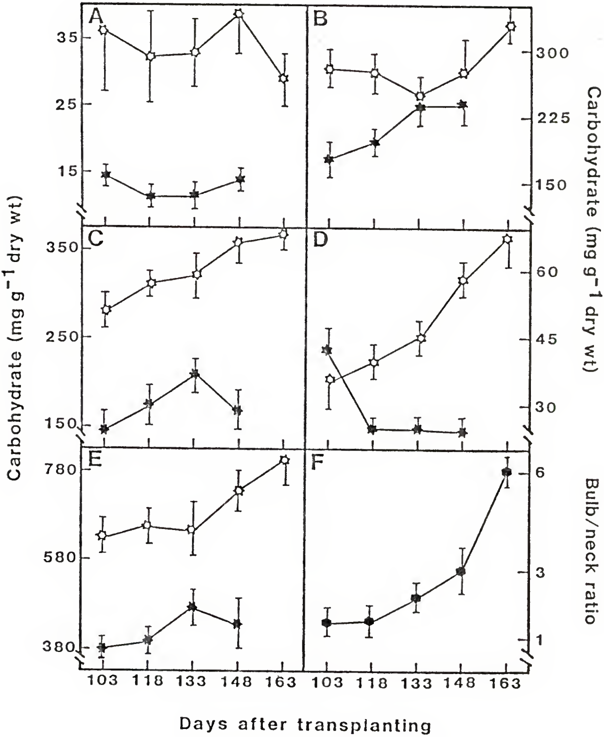

Upper section
V
4
3
2
1
103 days
outer leaves
Inner leaves
133 days
148 days
Lower section
103 days
133 days
148 days
Absorbance 540nm (relative units)
30
40
50
60
70
Fraction No
Fraction No

Fig. 3-3. Carbohydrate levels in leaves (✶) and bulbs (✡), and bulbing ratios (●) of the cultivar 'Granex 429' at different stages of development (days after transplanting). A, fructans; B, fructose; C, glucose; D, sucrose; E, total nonstructural carbohydrates; F, bulbing ratio.


A
B
C
D
E
F
35
25
15
300
225
150
350
250
150
60
45
30
780
580
380
6
3
1
103
118
133
148
163
103
118
133
148
163
Carbohydrate (mg g⁻¹ dry wt)
Carbohydrate (mg g⁻¹ dry wt)
Bulb/neck ratio
Days after transplanting

ratios 1.7 and 3.0. A sharp decrease in the levels of fructans was observed after the bulbs attained a bulbing ratio of 3.0. Fructose levels (B) decreased in the bulbs up to bulbing ratio 2.6 and then increased steadily. Glucose (C) and sucrose (D) in the bulbs increased throughout the experiment. The total nonstructural carbohydrate levels (E) also increased in the bulbs throughout their development, their rate of accumulation increasing markedly after bulbing ratio 2.4.

In the leaves (Fig. 3-3, A-E), quantitative changes in fructans (A) were small with a slight decrease up to bulbing ratio 2.4, then increasing again to initial levels. Fructose(B) and glucose(C) increased up to bulbing ratio 2.4, then either remaining constant (fructose) or decreasing (glucose). Sucrose levels (D) decreased sharply between bulbing ratios 1.3 and 1.7 showing no further changes in the subsequent dates. Total nonstructural carbohydrates in the leaves (E) accumulated up to bulbing ratio 2.4, then they decreased.

Plant part and location within a given plant part had a significant effect on the levels and distribution of fructans, fructose, glucose, sucrose and total nonstructural carbohydrates (Table A-1). The observed trends did not change over time. Fructans were higher in the bulbs where they increased centripetally and basipetally, whereas the opposite trends were observed in leaf blades (Fig. 3-4). The largest differences in fructan content were found in the lower part of the bulb.

Fructose levels were higher in bulbs than in leaves (Fig. 3-5). The outer scales and outer leaf blades contained more fructose than the inner scales and inner leaf blades. The largest differences were found in the lower part of the bulb (Fig. 3-5). Fructose content was higher in the upper portion than in the lower portion of the bulbs. The opposite was observed in the leaf blades.

Glucose levels were similar in the outer scales of upper and lower sections of bulbs (Fig. 3-6). The inner scales of upper sections had much more glucose than those of the lower sections. The lower leaf sections had much higher glucose levels than the upper with no difference between leaf position (Fig. 3-6).

Sucrose was higher in the bulbs than in the leaves (Fig. 3-7). There was more sucrose in the upper portion of the leaves than in the lower. The opposite was found in the bulbs (Fig. 3-7).

Total nonstructural carbohydrates were higher in bulbs than in leaves. Total nonstructural carbohydrate levels were slightly higher in the outer part of bulbs and leaf blades with significant differences found in the lower bulb only (Fig. 3-8).

Dry weight values were statistically different in bulbs and leaves, their levels also being affected by the location within the plant part (Table A-1). Dry weight

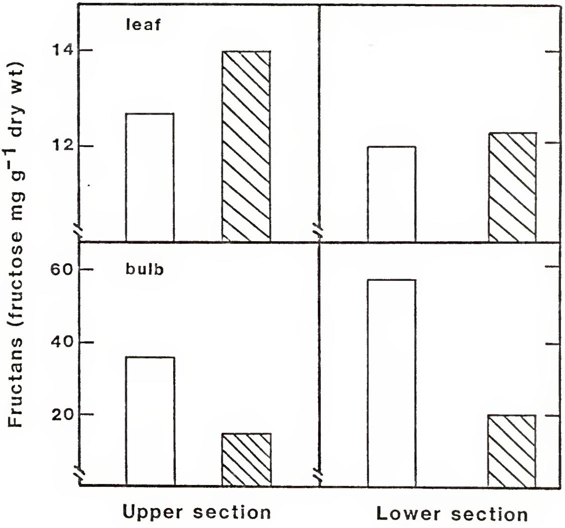


Fig. 3-4. Effect of plant part (leaf, bulb) and location within the plant part (upper, lower, inner, outer) on the levels of fructans of the cultivar 'Granex 429'. (□), inner; (▨), outer.

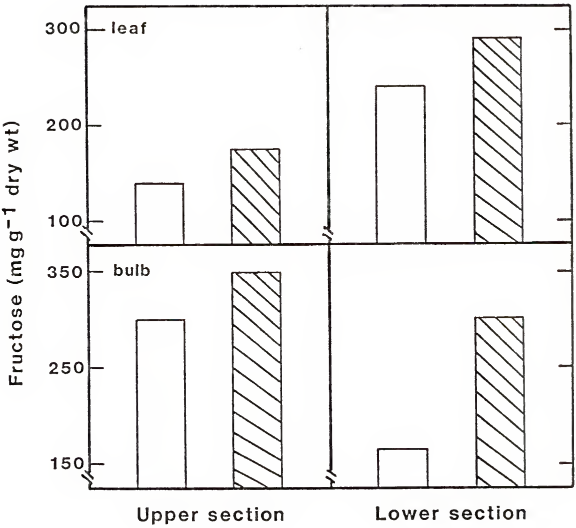


Fig. 3-5. Effect of plant part (leaf, bulb) and location within the plant part (upper, lower, inner, outer) on the levels of fructose of the cultivar 'Granex 429'. (☐), inner; (▨), outer.

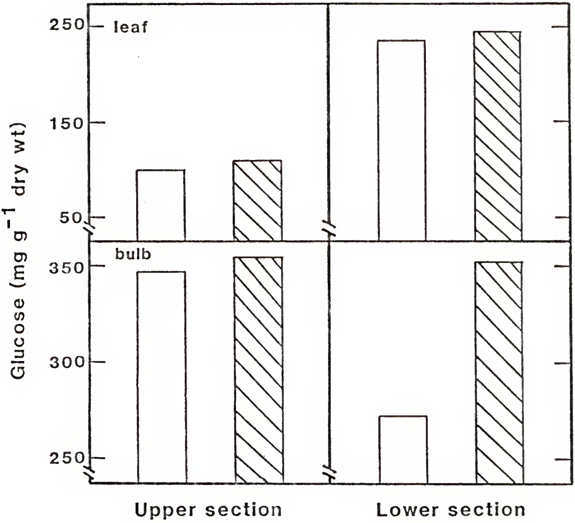


Fig. 3-6. Effect of plant part (leaf, bulb) and location within the plant part (upper, lower, inner, outer) on the levels of glucose of the cultivar 'Granex 429'. (□), inner; (▨), outer.

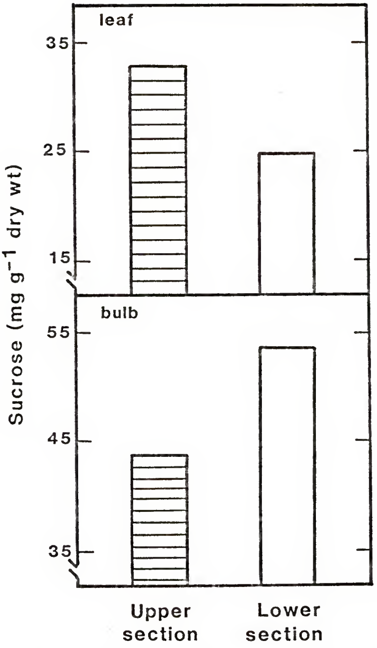


Fig. 3-7. Effect of plant part (leaf, bulb) and location within the plant part (upper, lower) on the levels of sucrose of the cultivar 'Granex 429'.

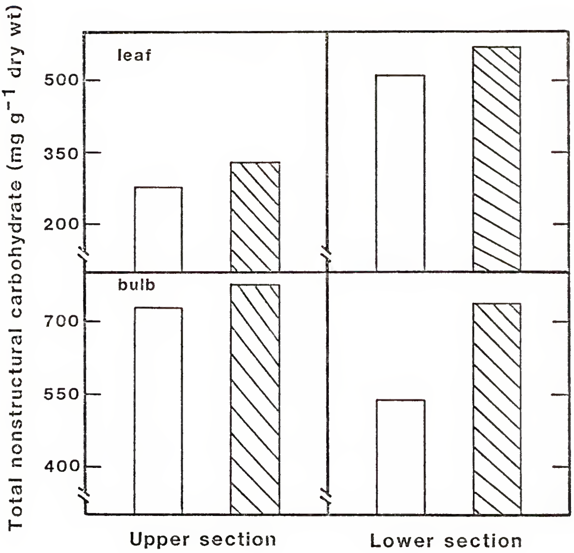


Fig. 3-8. Effect of plant part (leaf, bulb) and location within the plant part (upper, lower, inner, outer) on the levels of total nonstructural carbohydrates of the cultivar 'Granex 429'. (□), inner; (▧), outer.

levels were slightly higher in the inner bulb and leaves; the differences being significant in the lower bulb only (Fig. 3-9). Except for the dry weight of the inner and outer parts of the lower bulb, differences among other plant parts were rather small (Fig. 3-9).

Discussion

The molecular weight distribution of fructans found in the outer and inner scales of the bulb throughout its development (Fig. 3-1) was very similar to that of recently harvested mature bulbs (Chapter IV). The finding that longer chain fructans predominated differed from that reported for high latitude cultivars where the shorter degree of polymerization (DP) fructans usually predominate (Bacon, 1959b; Darbyshire, 1978; Darbyshire and Henry, 1978, 1979; Rutherford and Whittle, 1982). As mentioned in Chapter IV, these differences might have been partially due to differences in the techniques used for fructan extraction. In leaves, the presence of mainly DP3 and DP4 fructans (Fig. 3-2) agreed with the report by Darbyshire and Allaway (1981) for epidermal strips of onion leaf blades. Gas chromatography of the trimethylsilyl derivatives indicated the presence of mainly DP3 fructans in onion leaf blade samples (Darbyshire et al., 1979; Steer and Darbyshire, 1979).

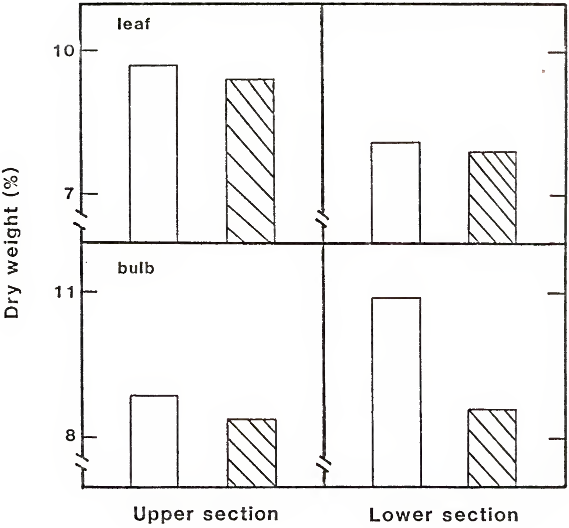


Fig. 3-9. Effect of plant part (leaf, bulb) and location within the plant part (upper, lower, inner, outer) on the dry weight of the cultivar 'Granex 429'. (□), inner; (▨), outer.

Carbohydrate levels changed over time (Fig. 3-3, A-E) and so did the bulbing ratio (Fig. 3-3, F). A bulbing ratio greater than 2 is usually taken to indicate bulbing, whereas ratios of 5 to 6.7 indicate fully developed bulbs (Mann, 1952; Clark and Heath, 1962). At 133 days after transplanting, the bulbing ratio value was slightly higher than 2 (Fig. 3-3, F) indicating the initiation of the stage of fast bulb growth. By that time there was a striking increase in the levels of fructans in the bulb (Fig. 3-3, A) which then decreased from 148 to 163 days coinciding with the highest rate of increase in bulbing ratio. The decrease in fructans as a percentage of the dry weight might have been due to the accumulation of other sugars at a higher rate during the last stages of bulb development. Levels of fructose, glucose and sucrose in the bulb increased at higher rates after 133 days (Fig. 3-3, B-D).

Fructan levels in the leaf blades decreased slightly before the bulb attained a bulbing ratio of about 2, increasing after that to its initial value (Fig. 3-3, A). Fructose and glucose accumulated in leaf blades up to 133 days (Bulbing ratio of about 2) remaining constant or decreasing after that, respectively, probably due to an increased mobilization of reserves to the growing bulb (Fig. 3-3, B-C). Sucrose seemed to be mobilized toward the base of the onion plant even before the establishment of bulbing as shown by the sharp decrease of its concentration levels in the leaf blades after the first sampling date (Fig. 3-3, D).

It has been shown in previous studies that during bulb formation sugars accumulate in all parts of the onion plant (Butt, 1968; Kato, 1965; Lercari, 1982b). In the present study, a marked increase in the concentration of total nonstructural carbohydrates was observed in the bulbs, especially after 133 days when the bulb started to develop at a high rate (Fig. 3-3, E). In the leaf blade, the level of total nonstructural carbohydrate increased steadily prior to the initiation of bulbing, then decreased slightly during the last sampling period (Fig. 3-3, E).

The quantitative distribution of fructans with higher values found in the bulbs and increasing centripetally (Fig. 3-4) was similar to the distribution pattern observed in high latitude cultivars (Bacon, 1959b; Darbyshire and Henry, 1978; Steer and Darbyshire, 1979). Henry and Darbyshire (1979) reported that fructosyltransferase activity increased from the outer to the inner scales whereas that of fructan hydrolase increased centrifugally. Working with a temperate grass (Dactylis glomerata), Pollock and Ruggles (1976) found that the levels and rates of fructan synthesis were much lower in leaf blades than in leaf bases. In the present experiment, fructans averaged about 5% of the total nonstructural carbohydrate fraction of the bulb and up to 10% in the lower inner part. These values are below the

20-50% found in corresponding tissue of high latitude onions (Darbyshire and Henry, 1978; Nilsson, 1980).

The markedly lower levels of fructans in the leaf blades (Fig. 3-4) are in agreement with reports for onions grown in other geographical areas (Steer and Darbyshire, 1979). However, direct comparison of the results from the present experiment and those of Steer and Darbyshire (1979) is not possible since those authors used juvenile plants in their experiments. Darbyshire et al. (1979) were not able to detect fructans in the leaf blades during daytime, and they estimated fructan levels from analyzed differences between total carbohydrates and individual sugars in extracts. In the present experiment fructans constituted about 3% of the total nonstructural carbohydrate fraction in leaf blades. Fructans in the leaf blades might play a role as a transient storage pool for any carbohydrate excess. Carbohydrate surplus can result when the rate of production is higher than its rate of use by the plant or plant part. Another reason would be the inability of the transport system to export or relocate the excess carbohydrate at a rate similar to that of its production in the leaf blade. Kemp and Blacklow (1980) studied the changes in carbohydrates in relation to leaf growth in wheat plants. They found the concentration of fructans in the extension zones increased from 0 to 11 mg g^{-1} fresh weight for younger and older leaves, respectively. They concluded that this was was due to an increasing over-supply of carbohydrates for growth as the leaves aged. Fructans in other monocotyledons have been induced to accumulate in leaves under conditions where the mobilization of sugars was altered (Blacklow et al., 1984; Wagner et al., 1983; Wagner and Wiemken, 1984).

Higher values of fructose in the bulbs (Fig. 3-5) might indicate accumulation of this sugar against a concentration gradient within the plant. The distribution of this sugar within each plant part could be partially related to the metabolism of fructans. There was an inverse relationship between the distribution of fructose and that of fructans (Fig. 3-4 and 3-5). This qualitative relationship was expected since fructose is the main product of fructan hydrolysis. The metabolism of sucrose and the possibility of reversible hexose isomerization might have contributed to the quantitative discrepancies found in the levels of these 2 carbohydrate fractions (Fig. 3-4 and 3-5). The trend of centrifugally increasing fructose concentration within the bulb is in agreement with that of high latitude cultivars (Darbyshire and Henry, 1978). The values of fructose found in bulbs and leaf blades were about 3 to 10 times higher than those reported for onions grown in other geographical areas (Darbyshire et al., 1979; Steer, 1982; Steer and Darbyshire, 1979).

Glucose distribution throughout the plant (Fig. 3-6) was very similar to that of fructose with the higher values found in the bulbs. This indicated an accumulation of this sugar against a concentration gradient. The values of glucose obtained in bulbs and leaf blades(Fig. 3-6) were also from 3 to 10 times higher than those previously reported (Darbyshire et al., 1979; Steer, 1982; Steer and Darbyshire, 1979). Glucose levels decreased centripetally within the bulb (Fig. 3-6) whereas Darbyshire and Henry (1978) found the values of this sugar tended to remain constant across the bulb in high latitude cultivars.

The decreasing levels of sucrose found from the upper to the lower parts of the leaf blades indicated a translocation pattern of this sugar towards the bulb where it seemed to accumulate to a greater extent in the lower part (Fig. 3-7). Steer and Darbyshire (1979) found that sucrose translocation to the leaf base occurred a short time after CO_2 fixation. The levels of sucrose found in the present experiment for bulbs and leaf blades (Fig. 3-7) were somewhat lower than those reported in high latitude cultivars (Darbyshire et al., 1979; Steer, 1982; Steer and Darbyshire, 1979). The lack of significant differences in the levels of sucrose of the outer and inner scales agrees with the findings of Darbyshire and Henry (1978) who observed that the levels of this sugar remained constant along the horizontal axis of the bulb.

The distribution of total nonstructural carbohydrates within the onion plant indicated a net accumulation of reserves in the bulb (Fig. 3-8). The leaf bases (scales) constitute sinks for carbon translocation both before and after bulb induction (Steer and Darbyshire, 1979). The levels of total nonstructural carbohydrates found in bulbs and leaves (Fig. 3-8) were in the range of those reported by Darbyshire et al. (1979) for juvenile onion plants.

The distribution of dry weight within the onion plant with the highest values found in the bulbs is in agreement with the accumulation of reserves in the developing bulb (Fig. 3-9). The dry weight distribution within the bulb resembled that of the fructans. There were no significant changes in the percentage dry weight over time which coincides with the results of Lancaster and Kathleen (1984). This means that the percentage dry weight of a young bulb is an accurate indication of the percentage dry weight of the mature bulb.

In summary, the results from this experiment showed that the onion bulb serves as a strong sink for carbohydrate accumulation. Fructose, glucose, sucrose and a series of fructan oligomers, constituted most of the nonstructural carbohydrate fraction of the bulb. Starch could not be detected in measurable amounts in the tissues analyzed which precludes it from being significant in the total nonstructural carbohydrate balance in quantitative terms. The translocation of reserves to the leaf bases seemed to occur throughout the growth of the onion plant but it reached a maximum after bulbing was induced. The carbohydrate concentration in bulbs was always higher than in the leaf blades; the difference became larger as bulbing progressed. This implied that movement of assimilates was against an apparent concentration gradient. Compartmentation of sugars in vacuoles, synthesis of fructans and sucrose hydrolysis in the bulbs might have contributed to this accumulation of reserves. The sugar content of the leaf blades is a net result of rate of sugar synthesis through photosynthesis and rate of consumption through respiration, utilization in growth, and translocation to the other plant organs. The observed increase in sugar content of leaves with age might have been due to the production of carbohydrates in excess of those consumed in respiration, growth, and translocated to other plant organs. Once bulb development was in progress, a large fraction of sugars moved from the blades to the developing bulb. This may explain the decrease of the sugar content in blades after reaching a maximum.

It is worth noting that the relative amounts of the sugars contributing to the total nonstructural carbohydrate fraction differed greatly between onions of the present experiment and those reported for cultivars grown in other geographical areas. Reducing sugars were predominant in onions from the present experiment whereas sucrose and fructans predominated in high latitude cultivars. This characteristic carbohydrate composition was extended to the storage period (Chapters IV and V). The molecular distribution of fructans in the bulbs throughout their development was similar to that found during storage

(Chapter IV). Larger DP chains predominated in the bulbs whereas the shorter ones are usually found in larger amounts in high latitude cultivars. In leaf blades, the fructan composition was qualitatively similar to that of onions grown in other geographical areas. The observed differences in the relative amounts of nonstructural carbohydrates throughout the development of the onion bulb might be related to the differences found in the storability of the bulbs from high and low latitutde cultivars.

CHAPTER IV
FRUCTAN CHANGES IN ONIONS DURING COLD STORAGE

Introduction

Due to specific photoperiodic requirements for bulbing, different onion cultivars are grown at different latitudes. Available information indicates that onions grown at high latitudes have more desirable storage characteristics (Brewster, 1977). Fructans are the major reserve oligosaccharides present in the onion bulb where, together with fructose, glucose and sucrose, they constitute the major nonstructural carbohydrate fraction (Bacon, 1957, 1959b; Bose and Srivastava, 1961; Darbyshire and Henry, 1978; Lolas and Markaris, 1973; Singh and Bhatia, 1972).

Any attempt to study the role of fructans in the storability of the cultivars grown at different latitudes would require basic information on the levels and changes of these carbohydrates during storage. These changes have been described for high latitude cultivars where fructans are unevenly distributed in the mature bulb with their concentration increasing centripetally (Bacon, 1959b; Darbyshire and Henry, 1978) and have a maximum degree of polymerization (DP) of about 12 (Darbyshire and Henry, 1978). In these cultivars, enzymic hydrolysis of the fructans takes place during storage (Rutherford and Whittle, 1982). Fructan levels and changes during storage have not been characterized for low latitude cultivars.

The present work describes the changes in content and degree of polymerization of the fructans in the bulbs of some low latitude onion cultivars during cold storage.

Materials and Methods

Effect of Bulb Size

Onions (*Allium cepa* L.) of 3 yellow cultivars 'F_1 Yellow Granex PRR'(YG); 'Texas Grano 502 PRR' (TEG-502) and; 'Texas Grano 1030Y' (TG-1030Y) were harvested on June 2, 1983 from a cultivar trial conducted at the Horticultural Unit in Gainesville. After curing for one week in a greenhouse at a maximum temperature of 32°C, with ventilation, the bulbs were sized into 2 categories: large (7-9 cm) and small (4-6 cm).

Effect of Cultivar, Growth Location and Growing Season

Bulbs of the yellow cultivar 'Granex 429' (G-429) were grown at the Hastings Agricultural Research and Education Center. The bulbs were harvested on May 20, 1983, and immediately transferred to the seed drying facilities of the Agronomy Department where they were dried for 2 days at 32°C with ventilation.

In a third experiment, onions of 2 yellow cultivars, G-429 and TG-1030Y were harvested on May 10 and 11, 1984 from the Horticultural Unit in Gainesville and Hastings Agricultural Research and Education Center, respectively. The bulbs were cured for 11 days at a maximum temperature of 32°C in a ventilated greenhouse. Bulbs of uniform size (5.5-7.5 cm) were used in both experiments.

Experimental Design

The experiments were complete factorial arrangements with time of storage as the main plot in a split-plot design with 3 replications.

Sampling for Storage

After curing, the sound bulbs were topped and the roots pruned. Onions were placed in mesh bags, weighed and stored at 1°C for 3 (Hastings, 1983) or 4 (Gainesville, 1983; Gainesville and Hastings, 1984) months. Relative humidity of the storage rooms ranged from 80% to 90%. Six bulbs per bag constituted the sample size in the Hastings 1983 experiment whereas 10 bulbs per bag were used for the other experiments. Weather conditions prevalent at harvest were more suitable (less relative humidity and less rainfall) in the Gainesville 1983 and Gainesville and Hastings 1984 crops resulting in drier and healthier bulbs that stored longer than those from the Hastings 1983 crop. *Pseudomonas sp.* and *Botrytis sp.* were determined to be the main causes of rotting during the storage trials.

Sampling for Analysis

Onions were sampled every 15 days. A longitudinal 1 cm. thick slice was taken across the center of the bulbs and both the outer (3 outermost) and inner (3 innermost) scales were analyzed separately for their fructan content. The dry weight of the outer and inner scales was also determined separately by drying the samples to a constant weight (65°C for 72 hours). The total number of scales per bulb ranged from 8 to 10.

Fructan Extraction and Analysis The preparation of the ethanol-insoluble powder (EIP) as well as the extraction and analysis of fructans were done as described in Chapter III.

Results

Small quantities of fructose, glucose and sucrose were consistently present in the powders and eluted at the degree of polymerization (DP) 1 and 2 positions of the P-2 profiles. Individually, these sugars never amounted to more than 2% of the corresponding values found for these carbohydrates in the ethanol-soluble fraction. Samples of the 90% ethanol-soluble filtrate applied to Bio-Gel P-2 contained only trace amounts of oligosaccharides (data not shown). These samples consisted primarily of fructose, glucose and sucrose. Further analysis of the trimethylsylil derivatives of the ethanol-soluble fraction revealed no measurable amounts of oligosaccharides of DP3 or greater.

The relative amounts of the different DP fructans were monitored every 15 days during the storage period. Typical qualitative results represented by the 1983 and 1984 G-429 crops are presented in Fig. 4-1 and 4-2. Similar results were obtained in the other cultivars in the corresponding years. Sampling dates not shown in the figures showed intermediate trends. Predominant at the beginning of the storage period, the longer-chain fructans exhibited a marked decrease relative to the shorter ones in the outer scales (Fig. 4-1 and 4-2). A less dramatic trend was observed in the inner scales during the first year (Fig. 4-1). Changes over time were less obvious in the inner scales during the second year (Fig. 4-2) where the longer DP oligosaccharides predominated throughout the storage period.

The levels of fructans found in the onion cultivars used in these experiments had mean values of 10 and 36 mg g^{-1} dry weight in the outer and inner scales, respectively. Fructans constituted between 1% and 5% of the total nonstructural carbohydrate fraction. Analysis of a sample from the onion cultivar 'Sentinel' grown in New York, yielded fructan levels of 107 and 208 mg g^{-1} of dry weight for the outer and inner scales, respectively.

FIG. 4-1. Bio-Gel P-2 chromatography of fructans from onion bulbs of the cultivar 'Granex 429' grown at Hastings in 1983 and stored at 1°C. Elution fractions for glucose, sucrose, stachyose, raffinose, and blue dextran are marked by I-V, respectively.

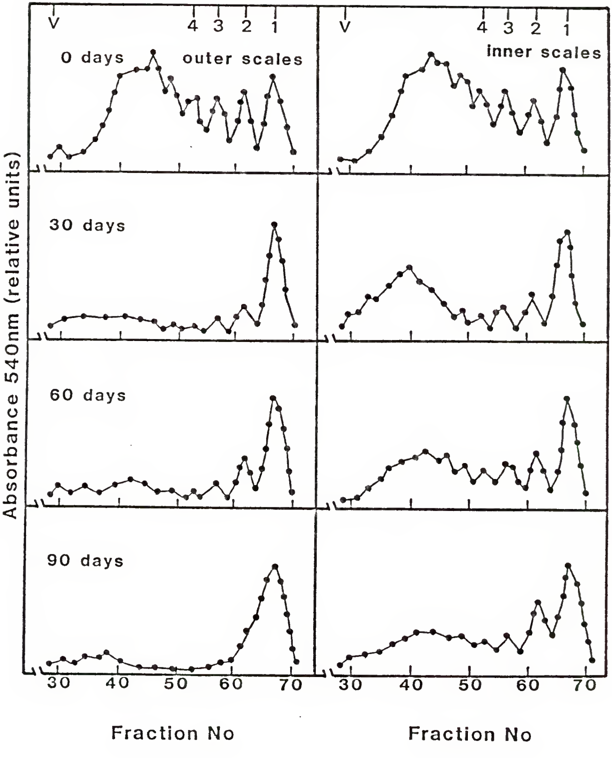
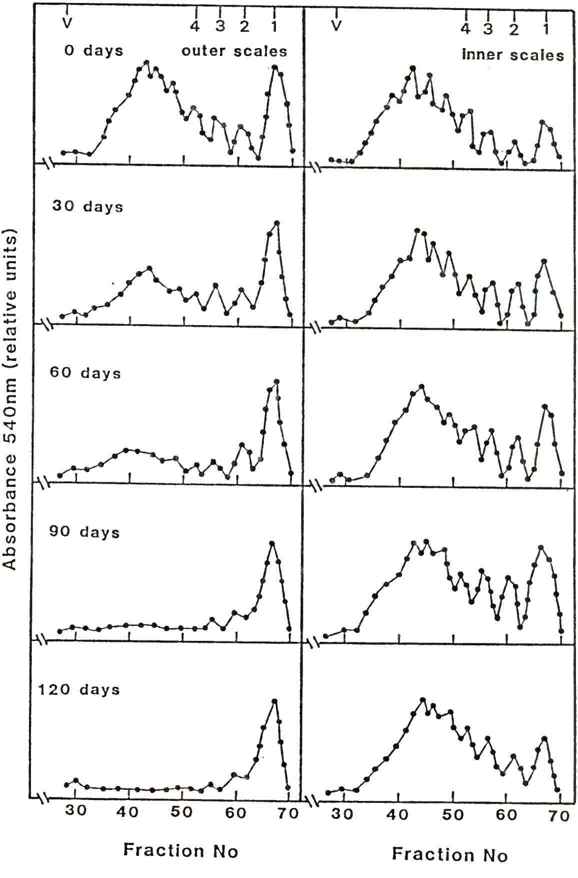

V
4 3 2 1
0 days
outer scales
V
4 3 2 1
inner scales
30 days
60 days
90 days
Absorbance 540nm (relative units)
30 40 50 60 70
30 40 50 60 70
Fraction No
Fraction No

Fig. 4-2. Bio-Gel P-2 chromatography of fructans from onion bulbs of the cultivar 'Granex 429' grown at Hastings in 1984 and stored at 1°C. Elution fractions for glucose, sucrose, stachyose, raffinose, and blue dextran are marked by I-V, respectively.


V
4 3 2 1
0 days
outer scales
inner scales
30 days
60 days
90 days
120 days
Absorbance 540nm (relative units)
30
40
50
60
70
Fraction No

Total fructan levels changed during storage. These changes were significantly influenced by the position of the scales within the bulb (Fig. 4-3, 4-4 and 4-5). There were no significant interactions between time of storage and cultivars, bulb size, or growth location (Table A-2, A-3 and A-4).

Fructan changes during storage were affected by growing seasons as shown by Fig. 4-3, 4-4 and 4-5. The differences were of greater magnitude in the inner scales. The outer scales had an overall decreasing trend in fructan content over time. Fructan levels were consistently higher in the inner scales with a decrease during storage in the first year (Fig. 4-3 and 4-4) but an increase after 60 days in storage in the second year (Fig. 4-5). The partitioning of these oligosaccharides within the bulb was significantly affected by growing seasons, growth locations, cultivars and bulb size (Fig. 4-6). In all cases, the differences were mainly due to the fructan levels of the inner scales whereas those of the outermost ones remained essentially unchanged (Fig. 4-6).

Dry weight changed significantly during storage (Table 4-1). These changes were not significantly affected by the location of the scales within the bulb during the first year (Table A-2 and A-3), but they were in the second year (Table A-4). Overall dry weight values were significantly affected by the growth location, the cultivars, and the location within the bulb (Table 4-2). The inner scales had

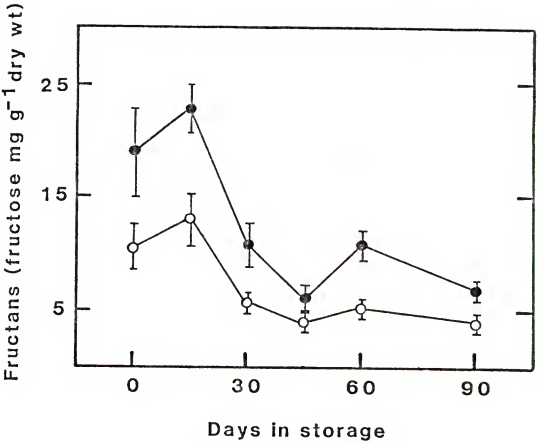


Fig. 4-3. Total fructans during cold storage (1°C) in bulbs of the onion cultivar 'Granex 429' grown at Hastings in 1983. Each point is the mean of 3 values, ± SE. (●), inner scales; (○), outer scales.

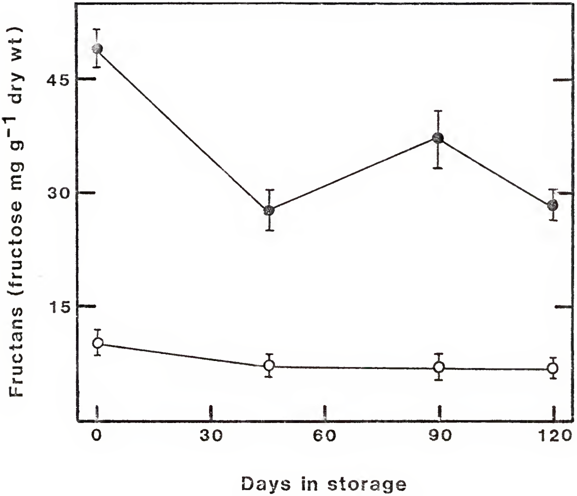


Fig. 4-4. Total fructans during cold storage (1°C) in bulbs of onions grown at Gainesville in 1983. Each point is the mean of 9 values, ± SE. (●), inner scales; (○), outer scales.

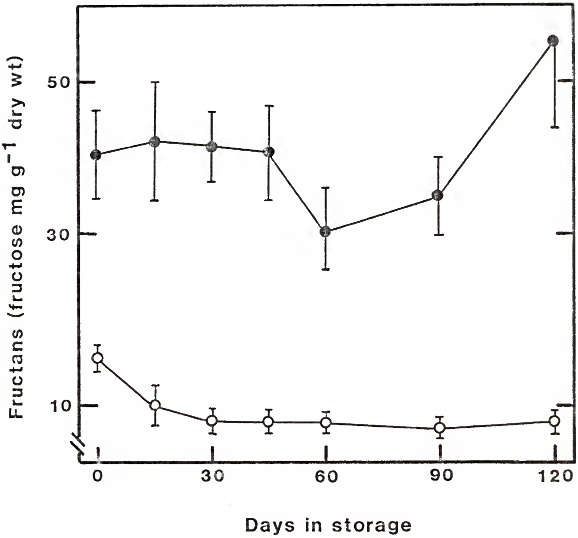


Fig. 4-5. Total fructans during cold storage (1°C) in bulbs of onions grown at Hastings and Gainesville in 1984. Each point is the mean of 12 values, ± SE. (●), inner scales; (○), outer scales.

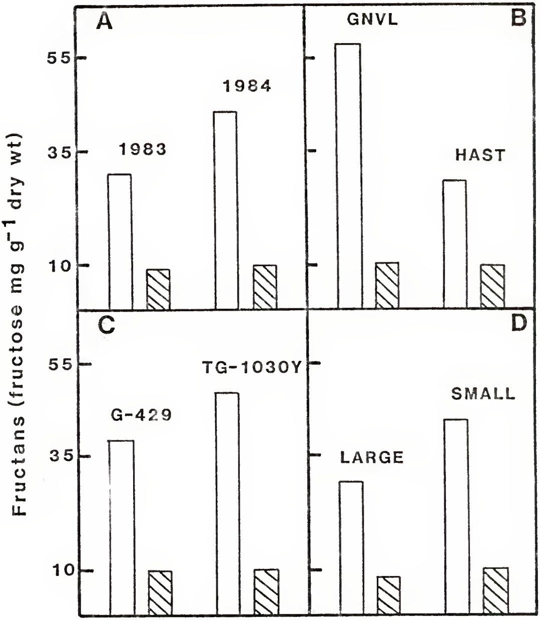


Fig. 4-6. Effect of growing season (A), growth location (B), cultivar (C), and bulb size (D) on the fructan content of onion bulbs stored at 1°C. (□), inner scales; (▨), outer scales.

Table 4-1. Dry weight of onion bulbs during storage at 1^{o}C. Data are means of 6 (1983) and 12 (1984) observations, respectively.

Days in Storage	1983[1] HASTINGS	1983[1] GAINESVILLE	1984[2] OUTER	1984[2] INNER
	% DRY WEIGHT			
0	8.7	10.3	9.0	10.5
15	9.0	10.2	8.3	9.7
30	8.7	9.7	8.4	9.6
45	8.9	10.3	8.3	10.6
60	9.1	9.7	8.3	10.7
90	8.2	8.5	8.2	10.8
120	___	9.4	8.2	11.2

---

[1] Outer and inner scales combined.
[2] Combined for growth locations.

Table. 4-2. Effect of growth location, cultivar, and location within the bulb on the dry weight of onion bulbs grown in 1984 and stored for 4 months at 1°C. Data are means of 21 observations.

Growth location	Cultivar	Location within the bulb	
		outer	inner
		.... % DW	
Gainesville	G-429	8.5	10.0
	TG-1030Y	9.0	11.0
Hastings	G-429	7.7	9.0
	TG-1030Y	8.4	10.5

higher dry weight than the outer. The cultivar TG-1030Y had a higher dry weight than G-429. Bulbs grown in Gainesville had a higher dry weight than those grown in Hastings.

Discussion

The 90% ethanol-insoluble powders (EIP) was the primary fructan source as shown by the positive reaction with resorcinol either before or after separation of the oligomers in Bio-Gel P-2 and by the almost complete absence of these carbohydrates in the ethanol-soluble fraction. This technique resulted in a more quantitative recovery of the longer degree of polymerization (DP) chains as compared to that of previous reports (Darbyshire, 1978; Darbyshire and Henry, 1978, 1979, 1981; Rutherford and Whittle, 1982) where the 80% ethanol soluble fraction was used as the source of fructans. The fructans values (107-208 mg g^{-1} dry weight) found in this study for the onion cultivar 'Sentinel' were in the range of those reported for other high latitude cultivars (Bacon, 1959b; Darbyshire and Henry, 1978, 1979; Rutherford and Whittle, 1982) which showed that the technique used in the present experiments for the extraction of fructans was adequate.

The molecular weight distribution of fructans found in these experiments (Fig- 4-1 and 4-2) did not always resemble those reported for high latitude cultivars where the shorter fructans (DP3 and DP4) usually predominate (Bacon, 1959b; Darbyshire, 1978; Darbyshire and Henry, 1978, 1979; Rutherford and Whittle, 1982). According to Darbyshire and Henry (1979), recently harvested high-dry-weight cultivars contained high concentrations of longer DP fructans (maximum concentration at DP6). The onions from the present experiments fell into the category of "medium" reported by those authors regarding percentage dry weight (about 9 to 13% dry weight) . However, the molecular weight distribution of fructans at 0 days storage more closely resembled that of "high" dry weight cultivars (about 14 to 21% dry weight) found by Darbyshire and Henry (1979). Maximum values of fructans at DP5 were found in a bunching onion cultivar (Darbyshire and Henry, 1981) whereas those of the present experiments had their maximum values at DP6-7 (Fig. 4-1 and 4-2). Darbyshire and Henry (1978) estimated that DP3 was the dominant polymer. The differences in the relative distribution of the different molecular weight fructans between the cultivars used in the present experiments and those from earlier studies (Bacon, 1959b; Darbyshire, 1978; Darbyshire and Henry, 1978, 1979; Rutherford and Whittle, 1982) might have been partially due to differences in the techniques used for fructan extraction.

The levels of fructans (10-36 mg g^{-1} dry weight) found in the onion cultivars used in the present experiments were much lower than those reported for high latitude cultivars (Bacon, 1959b; Darbyshire, 1978; Darbyshire and Henry, 1978, 1979; Rutherford and Whittle, 1982). Fructans constituted a very small fraction of the total nonstructural carbohydrate content (1-5%) compared to the 17 to 33% found in New York grown 'Sentinel' and 20-50% reported for another high latitude cultivar (Darbyshire and Henry, 1978). The differences found in the size of the fructan pools in low and high latitude cultivars indicates a possible role for these carbohydrates in the storability of onions. In the present experiments, bulbs with higher fructan content appeared to have more desirable storage characteristics as indicated by sprouting, decay incidence, weight loss and firmness (Toledo et al., 1984, 1985). Although fructan levels were significantly different for large (19 mg g^{-1} dry weight) and small (26 mg g^{-1} dry weight) bulbs, this did not seem to correlate with any storage differences of commercial significance other than the small bulbs being firmer (Toledo et al., 1984).

The uneven distribution of fructans within the bulb (Fig. 4-6) is in agreement with that reported in high latitude cultivars (Darbyshire and Henry, 1978). These findings are also consistent with the different activities of the enzymes involved in the synthesis and hydrolysis of fructans in different scales (Henry and Darbyshire, 1978, 1979). These authors reported that the activity of sucrose:sucrose fructosyl transferase (SST) increased centripetally while fructan hydrolase activity decreased in bulbing onions.

The decrease in fructan content of the outer scales (Fig. 4-1 to 4-5) during storage indicates that these oligosaccharides were subjected to hydrolysis during cold storage. Hydrolysis of fructans to free fructose and sucrose has been proposed to be enhanced by low temperatures (3-5^{o}C) during storage in high latitude onions (Darbyshire, 1978; Rutherford, 1981; Rutherford and Whittle, 1982) as well as for other fructan-containing species (Bacon and Loxley, 1952; Haaland, 1974; Haaland and Wickstrom, 1975; Jefford and Edelman, 1961, 1963; Moe and Wickstrom, 1973; Rutherford and Weston, 1968).

Fructan levels decreased in the inner scales during storage (Fig. 4-3 and 4-4) but this trend was reversed in the second year after 60 days (Fig. 4-5) suggesting the possibility of differences in the metabolic activity of the stored bulbs between the first and second year trial. During 1983, the bulbs showed rudimentary signs of sprout development as shown by the yellowing of the innermost scales (Tucker, 1971). This became more evident as storage progressed. No signs of sprouting were observed in the 1984 experiments where only the appearance of new roots was observed with time. In addition to partially providing the necessary substrate to reactivate metabolism in the sprouting region, the hydrolysis of the fructans may have helped adjust their osmoticum to favor the development of the necessary turgor for the resumption of growth. Besides being reserve carbohydrates (Halmer and Bewley, 1982; Hirst, 1957; Kandler and Hopf, 1982; Meier and Reid, 1982; Pollock and Ruggles, 1976), it has been suggested that fructans play a role in providing osmotic adjustment in meristematic areas (Nelson and Coutts, 1984). A similar function has been proposed for these oligomers during the bulbing process of onions (Darbyshire and Henry, 1978; Henry and Darbyshire, 1979).

Factors such as cultivar, growth location, bulb size and growing season had a much greater effect in the levels of fructans in the inner scales (Fig. 4-6). Environmental effects on fructan metabolism have been reported for other species (Del Campillo and Pontis, 1984; Eagles, 1967; Nelson and Coutts, 1984; Pollock, 1981; Smith, 1968; Smith, 1977). No significant effects of growth temperatures on the fructan content of onions was found by Steer (1982).

The distribution of dry weight within the bulb resembled that of the fructans (Table 4-2). The observed increase in dry weight from the outer to the inner scales was similar to that previously reported (Darbyshire, 1978; Darbyshire and Henry, 1979; Mann and Hoyle, 1945). Changes over time resembled those of the fructans (Table 4-1).

In summary, the results from these experiments showed that fructans in the onion bulb changed both qualitatively and quantitatively during cold storage and that these changes were different in the outer and inner scales. The molecular weight distribution of the fructan oligomers in low latitude onions differed from that reported for high latitude cultivars. Longer DP chains predominated and decreased during storage in the outer scales only. The overall decrease in fructan content in the outer scales over time was similar to that observed in high latitude cultivars. This trend was less evident in the inner scales. Fructan levels were not significantly affected by cultivar, bulb size or growth location but the trends differed over seasons. Total fructan levels were much lower than those found in high latitude cultivars.

Differences in the fructan pools of onion cultivars from different latitudes and the trends in the changes of these oligomers during storage suggests a role in the storability of the bulbs. Although these data are not conclusive, a possible role for fructans as being osmoregulators is suggested. This needs to be investigated further. Changes in the activities of the enzymes involved in the synthesis and hydrolysis of fructans and their distribution within the bulbs throughout storage also need to be studied to confirm and explain the observed changes and their physiological role.

CHAPTER V
METABOLISM OF FRUCTOSE, GLUCOSE AND SUCROSE IN ONIONS DURING COLD STORAGE

Introduction

Numerous attempts have been made to correlate physiological and biochemical factors to the storage longevity in onion bulbs. Good storability has been related to high dry matter content of the bulbs (Foskett and Peterson, 1950) and to long photoperiod during bulb maturation (Isenberg, 1979). The exact performance in terms of length of storage varies from season to season and, in this regard, the dry matter values cannot be used to forecast differences in the expected storage duration following a given growing season (Rutherford and Whittle, 1984).

One of the most important biochemical changes occuring during cold storage of vegetables are in the carbohydrate constituents (Rutherford, 1977). Kato (1966) concluded that the metabolism of sugars was closely linked with bulb dormancy in onions. The main sugars present in onion bulbs are fructose, glucose and sucrose together with a series of oligofructosides (Bacon, 1957, 1959b; Bose and Srivastava, 1961; Butt, 1968; Darbyshire and Henry, 1978; Lolas and

Markaris, 1973; Singh and Bhatia, 1972). In spite of the importance of these carbohydrates in the metabolism of the stored bulb, very little information is available concerning the changes in content of the individual sugars during storage. Darbyshire (1978) drew attention to increases in amounts of fructose during cold storage whereas Rutherford and Whittle (1982, 1984) indicated that the amounts of fructose in recently harvested onions together with the activity of alkaline invertase in the stored bulbs could be related to their storage longevity. The above mentioned studies involved high latitude cultivars. Low latitude onions are known not to be good keepers (Smittle and Williamson, 1978; Pike and Leeper, 1982) and this has been attributed partially to their relatively low solids (Smittle et al., 1979). Hurst et al. (1985) have recently studied the changes in total sugars during storage of low latitude onions. They found that the level of total sugars increased in bulbs stored at 4°C. Total sugars levels remained fairly constant after 4 weeks of storage at 1°C.

The objective of this work was to study the changes in fructose, glucose and sucrose in relation to the fructan changes during cold storage of some low latitude onion cultivars.

Materials and Methods

Details regarding the growing of the crops, experimental design, storage conditions and sampling methods were described in Chapter IV. Sugar extraction and analysis were done as in Chapter III.

Results

Over 98% of the individual content of fructose, glucose, and sucrose, respectively, were found in the 90% ethanol soluble fraction. The corresponding analysis of variance tables for the 3 experiments are presented in the Appendix (Table A-2, A-3, A-4).

Fructose

The levels of fructose were significantly higher in the outermost scales than in the inner (Fig. 5-1). The distribution of fructose within the bulb was affected by the size of the bulb, the cultivar, and the growth location (Fig. 5-1, A-C). The large difference between the inner scales were primarily responsible for the interactions between cultivars (B) and growth locations (C).

Fructose levels changed significantly during storage (Fig. 5-2, A-C). Fructose content increased during storage in the first year experiments (Fig. 5-2, A-B) whereas a decreasing trend was observed in the second year experiments (Fig. 5-2, C). Fructose constituted about 50%

Fig. 5-1. Effect of bulb size (A), cultivar (B), and growth location (C) on the distribution of fructose in the inner (☐) and outer (▧) scales of onion bulbs stored at 1°C.

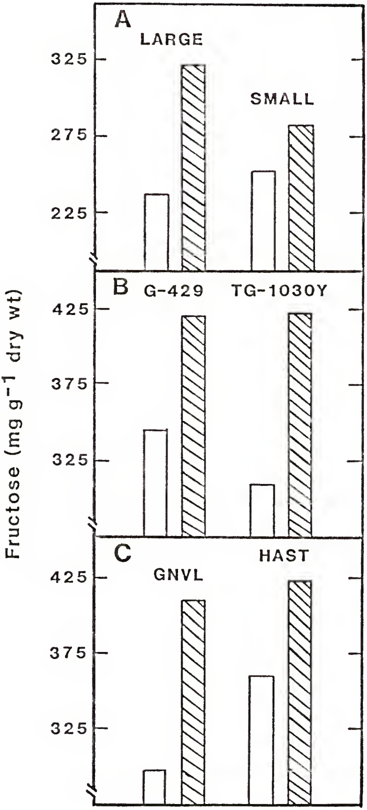
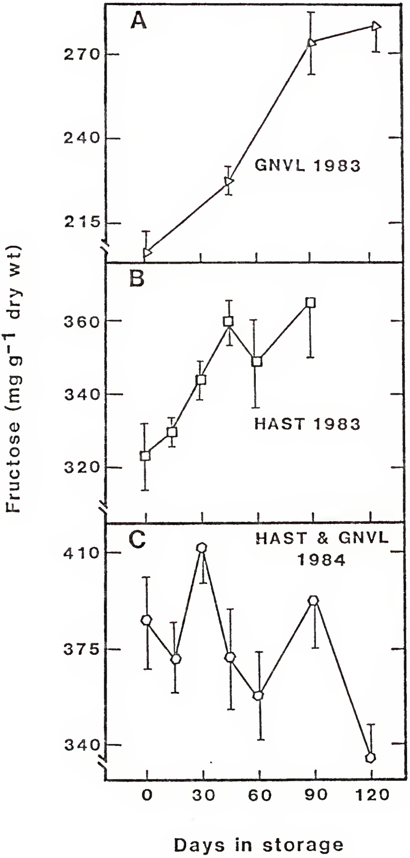

A
LARGE
SMALL
325
275
225
B
G-429
TG-1030Y
425
375
325
C
GNVL
HAST
425
375
325
Fructose (mg g^{-1} dry wt)

Fig. 5-2. Fructose changes during cold storage ($1^{\circ}C$) in onion bulbs. Each point is the mean of 18 (A), 6 (B), and 24 (C) values ± SE, respectively.


A
B
C
GNVL 1983
HAST 1983
HAST & GNVL
1984
270
240
215
360
340
320
410
375
340
0
30
60
90
120
Fructose (mg g⁻¹ dry wt)
Days in storage

of the reducing sugars and 44% of the total nonstructural carbohydrates (fructose, glucose, sucrose, and fructans).

Glucose

Values of glucose were significantly higher in the outermost scales than in the inner (Fig. 5-3). The levels of this sugar within the bulb were significantly affected by the size of the bulb and the growth location (Fig. 5-3, A-B). The differences between the inner scales were primarily responsible for the interaction between locations.

Changes in glucose content during storage varied among experiments. (Fig. 5-4, A-C). It decreased in the Gainesville 1983 (A) and Gainesville and Hastings 1984 (C) experiments. A less definite trend was observed in the Hastings 1983 (B) experiment where glucose levels decreased after 15 and 45 days in the inner and outer scales, respectively. Quantitative changes over time were statistically different within the bulb for the first year experiments (Table A-2, A-3). Growth location had an effect on the changes of glucose during storage in the third experiment (Table A-4). Glucose comprised about 50% of the reducing sugars, and 45% of the nonstructural carbohydrates.

Sucrose

The levels of sucrose were significantly higher in the inner scales than in the outer (Fig. 5-5). The values of sucrose found in the onion cultivars during 1984 were

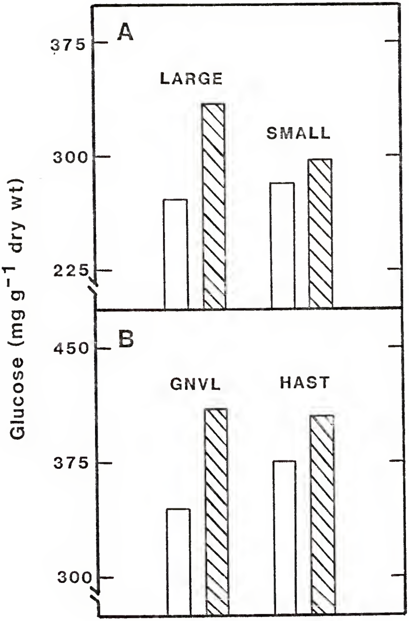


Fig. 5-3. Effect of bulb size (A) and growth location (B) on the distribution of glucose in the inner (☐) and outer (▧) scales of onion bulbs stored at 1°C.

Fig. 5-4. Glucose changes during cold storage (1°C) in onion bulbs. Each value is the mean of 9 (A), 3 (B), and 12 (C) ± SE, respectively. In A and B, (●), inner scales; (○), outer scales. In C, (▲) Hastings; (■) Gainesville.

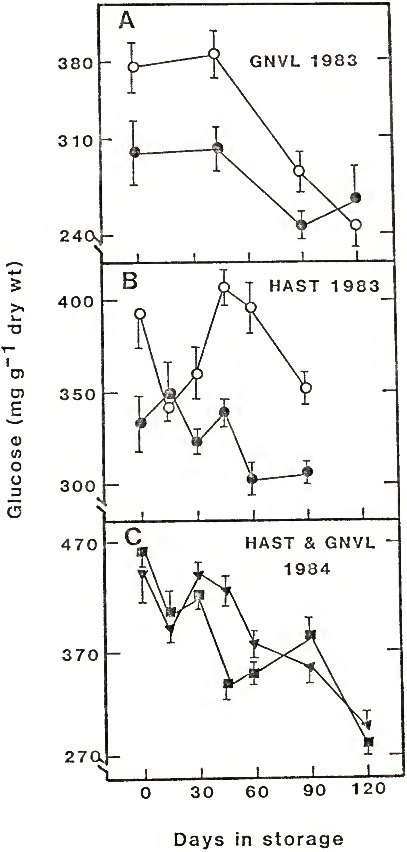
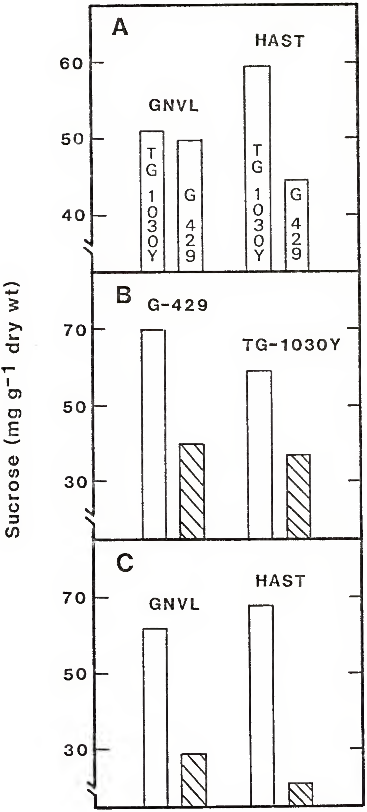

A
GNVL 1983
380
310
240
B
HAST 1983
400
350
300
C
HAST & GNVL
1984
470
370
270
0
30
60
90
120
Glucose (mg g⁻¹ dry wt)
Days in storage

Fig. 5-5. Interaction effects on sucrose levels of onion bulbs stored at 1°C. Effect of growth location (A) on the levels of sucrose in the bulbs of 2 onion cultivars 'Texas Grano 1030Y' and 'Granex 429'. Effect of cultivar (B) and growth location (C) on the distribution of sucrose in the inner (☐) and outer (▨) scales.

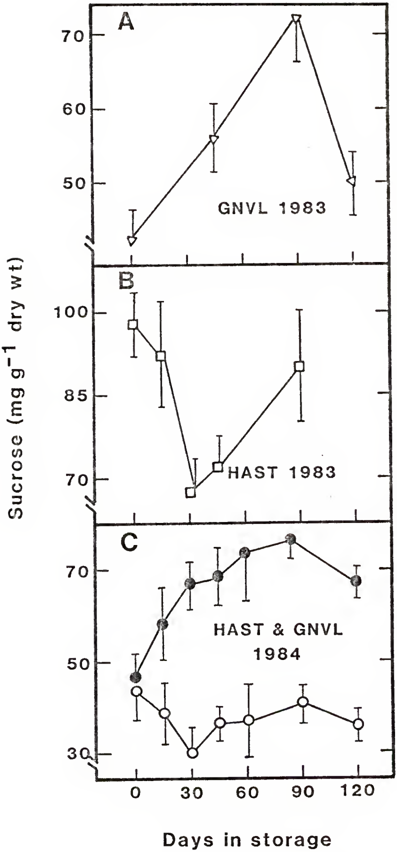

A
HAST
GNVL
60
50
40
TG 1030Y
G 429
TG 1030Y
G 429
B
G-429
TG-1030Y
70
50
30
C
HAST
GNVL
70
50
30
Sucrose (mg g⁻¹ dry wt)

influenced by the growth location (Fig. 5-5, A). The distribution of the sugar within the bulb was sigificantly affected by the cultivar and the growth location (Fig. 5-5, B-C). Sucrose changes during storage were different among experiments (Fig. 5-6, A-C). A linear increase in sucrose was observed in the Gainesville 1983 experiment (A) up to 90 days in storage followed by a decrease. In the Hastings 1983 experiment (B) this trend was reversed with sucrose levels decreasing during the first 30 days of storage and then increasing steadily. In these 2 experiments, the quantitative changes in sucrose over time were not affected by the position of the scales within the bulb (Table A-2, A-3). In the second year experiments (C), changes in sucrose during storage were significantly different within the bulb with a trend toward increasing levels in the inner scales with decreasing levels in the outer ones. Sucrose was about 8% of the total nonstructural carbohydrate fraction.

Discussion

The decrease in the levels of fructose (Fig. 5-1, A-C) and glucose (Fig. 5-3, A-C) toward the inner scales is in agreement with that reported for high latitude onions (Bacon, 1959b; Darbyshire and Henry, 1978). The observed higher values of fructose in the outermost part of the bulb seemed to support previous reports that fructans are

Fig. 5-6. Sucrose changes during cold storage (1°C) in onion bulbs. Each point is the mean of 18 (A), 6 (B), and 12 (C) values, ± SE, respectively. In C, (●) inner scales; (○) outer scales.


A
GNVL 1983
70
60
50
B
HAST 1983
100
85
70
C
HAST & GNVL
1984
70
50
30
0
30
60
90
120
Sucrose (mg g^{-1} dry wt)
Days in storage

hydrolyzed at a higher rate in the outer scales (Darbyshire and Henry, 1978). Hydrolysis of sucrose and hexose isomerization might also have contributed to the observed fructose and glucose values. The mean levels of fructose (Fig. 5-1 and 5-2) and glucose (Fig. 5-3 and 5-4) found in these experiments were higher than those found by Rutherford and Whittle (1982, 1984). In both outer and inner scales, the contribution of fructose (44%) and glucose (46%) as percentage of the nonstructural carbohydrate fraction was very similar. These values were higher than those given by Darbyshire and Henry (1978) which ranged between 8% and 30% for fructose and from 23% to 28% for glucose in the outer and inner scales, respectively.

The higher sucrose values of the inner scales as compared to the outer scales (Fig. 5-5, A-C) agrees with the results obtained for onions grown in other geographical areas (Darbyshire, 1978; Rutherford and Whittle, 1982) but disagrees with Bacon (1959b) who found that sucrose levels fell toward the center of the onion bulb. This uneven distribution of sucrose within the bulb could have been due to the transport of this sugar to the innermost scales to sustain the new growth. Higher rates of hydrolysis of sucrose in the outer scales might have also contributed to the lower values obtained in that part of the bulb. The mean levels of sucrose found in the present experiments for the outer (53 mg g^{-1} dry weight) and inner (72 mg g^{-1}

dry weight) scales were lower than those from high latitude onions (Darbyshire, 1978; Rutherford and Whittle, 1982). It was found that sucrose comprised only 8% of the nonstructural carbohydrate fraction compared to the 20-30% values found by Darbyshire and Henry (1978).

Bulb size had an effect on the distribution of fructose (Fig. 5-1, A) and glucose (Fig. 5-3, A) within the bulb. The difference in the levels of fructose and sucrose of the outer and inner scales was larger in the larger bulbs. Hurst et al. (1985) also found that the levels of sugars were affected by different bulb sizes. However, sucrose distribution was not significantly affected by the size of the bulb (Table A-2). In a storage trial conducted by Toledo et al. (1984) with the onions from the present experiments, it was found that different bulb sizes showed no storage differences of commercial importance.

There was a significant effect of cultivars on the distribution of fructose (Fig. 5-1, B) and sucrose (Fig. 5-5, B) within the bulb. The differences were more evident in the inner scales, with G-429 having higher values of fructose and sucrose than TG-1030Y. This higher accumulation of sugars in the inner part of the bulb of cultivar G-429 could have been related to an earlier resumption of growth. Toledo et al. (1985) found G-429 to have significantly higher root sprouting during storage than TG-1030Y. Kato (1966) observed that carbohydrates were translocated toward the inner scales prior to shoot sprouting.

Growth locations had an effect on the distribution of the 3 sugars within the bulb (Fig. 5-1, C; 5-3, B; 5-5.C). Onion bulbs grown at Gainesville had lower levels of the individual sugars in the inner scales. Gainesville bulbs stored better then those from Hastings (Toledo et al.,1985).

Sugar levels varied during storage. The differences observed among experiments could have been influenced by the growing conditions (Isenberg et al., 1974; Rutherford and Whittle, 1982). Gorin (1982) found that the pattern of the individual sugars was not reproducible in a 2-year trial. The increase in fructose during storage in the first year experiments (Fig. 5-2, A-B) may have been due, in part, to the hydrolysis of fructans observed throughout the bulb over time (Chapter IV). This is in agreement with previous reports for high latitude cultivars (Darbyshire, 1978; Rutherford and Whittle, 1982). Similar results have also been found for other fructan-containing species (Davies and Kempton, 1975; Deacon and Rutherford, 1972; Edelman and Bacon, 1951; Jefford and Edelman, 1963). The increase in fructose during storage is interpreted as the need for substrate to sustain new growth. As mentioned in Chapter IV, incipient signs of shoot sprouting were observed as storage progressed in the first year experiments.

In general, fructose levels decreased during storage in the second year (Fig. 5-2,C) indicating possible differences in the metabolism of the bulbs compared to the first year experiments. Less fructan hydrolysis in the 1984 crops, especially in the inner scales (Chapter IV), together with the transport of fructose to the growing roots might have contributed to the decrease in fructose values in the bulbs over time. The first large drop in fructose after 30 days of storage (Fig. 5-2, C) coincided with the appearance of the first signs of rooting (Toledo et al., 1985). The second drop in fructose after 90 days storage paralleled the rapid increase in size of the roots. No visual signs of shoot sprouting were observed in the 1984 storage trials. Although the observed changes in fructose during storage corresponded qualitatively to those of the fructans (Chapter IV), the large quantitative differences in the observed values of the 2 carbohydrate pools indicated that changes in other sugars must have contributed to their observed values. Linear correlation coeficients for fructans and fructose in the three experiments (-0.432, -0.691, -0.559)showed a weak inverse linear relationship between these 2 parameters. Sugar translocation within the bulb, hexose isomerization and respiration might have added to the complexity of the carbohydrate balance of the bulb. Similarly, quantitative discrepancies in the carbohydrate changes during storage have been reported previously for other species.

Rutherford and Weston (1968) found that the amount of total ethanol-soluble sugars did not vary proportionally with changes in the higher molecular weight sugars of roots of chicory and dandelion and tubers of Jerusalem artichoke. Moe and Wickstrom (1973) found the concentration of fructose and glucose showed slight and irregular variations in tulip bulbs kept at either 5°C or 21°C. The contribution of fructans to the observed changes in reducing sugars was negligible.

The only reports on carbohydrate changes in onions during storage in relation to fructan metabolism are those of Darbyshire (1978) and Rutherford and Whittle (1982). These involved onions grown in other geographical areas. Although direct comparisons of their experiments and those presented here are not possible because of the different techniques used for the extraction of fructans, some observations on their methods and conclusions are appropiate. Darbyshire (1978) said that the increase in fructose levels in bulbs during cold storage was due to the hydrolysis of fructans. However, no data were presented to support this statement. The only fructan fraction he was able to estimate was the trisaccharide, which according to his data showed no variation during the storage trial (3 months). Besides, fructose changes during storage and the corresponding ones of the trisaccharide fructan fraction were assessed in different scales within the bulb, making comparisons difficult. Finally, conclusions on the changes of the total fructan fraction cannot be based on the variations observed in just one of its components (trisaccharide). Sample size was not indicated in this paper and, according to the author, the results were not handled statistically.

Rutherford and Whittle (1982) concluded that the main change observed during storage of onions was the hydrolysis of fructans to reducing sugars. They measured changes in fructose, glucose and sucrose in 8 individual scales within the bulb. Fructans up to degree of polymerization (DP) 5 were quantitated in scales 3 and 7 only as representatives of the outer and innermost scales, respectively. In their first experiment, the increase in fructose during storage might have accounted for up to about 50% of the reduction in fructan levels in scale 3. Glucose levels decreased about 20% in the same scale during this experiment. In the second trial fructose levels might have accounted for about 75% of the fructans hydrolyzed in scale 3. Glucose levels did not change during storage during the second year experiment. In scale 7, fructose might have accounted for only 55-60% of the fructan changes in the 2 years. Glucose decreased by 20% in scale 7 during the first year trial whereas it changed only slightly during the second year. The actual differences between fructan changes and reducing sugars might have been even larger considering that their fructan extraction technique (80% ethanol-soluble fraction) may have underestimated the amounts of the larger DP chains as mentioned in Chapter IV.

Fructans are soluble in water, insoluble in absolute alcohol, and their solubility in aqueous alcohol depends on the proportion of water present (Archbold, 1940). Wylam (1954) found only short-chain fructans in 80% ethanol extracts of ryegrass. Working with *Dactylis glomerata*, Eagles (1967) used the 80% ethanol extract as the source of simple sugars. The ethanol-insoluble powder (EIP) resuspended in water was used for determination of fructans. Several authors working with different species found the most accurate separation of simple sugars from fructans with 90-95% ethanol (Chen and Hou, 1981; Edelman and Dickerson, 1966; Holligan et al., 1973; Khodzhaeva and Ismailov, 1979; Smith, 1967, 1968; Smith and Grotelueschen, 1966; Grotelueschen and Smith, 1968; Volenec and Nelson, 1984a,b). Low temperature treatment of the ethanol-extracted carbohydrate mixture has been used to enhance the precipitation of the ethanol insoluble substances (Edelman and Dickerson, 1966).

Critical examination of the data and methods of Darbyshire (1978) and Rutherford and Whittle (1982) makes it unlikely that changes in fructans during storage of onions can be completely accounted for by the corresponding changes in fructose. Synthesis and hydrolysis of sucrose and glucose, hexose isomerizarion, translocation and respiration are factors that add to the complexicity of the overall carbohydrate balance of the onion during storage.

Changes in glucose during storage more or less resembled those found for fructose in the 1984 experiments (Fig. 5-4, C). The interaction between time of storage and growth location was significant (Table A-4) showing opposite trends in the changes of glucose between 45 and 90 days in Gainesville and Hastings. The overall trend, however, was similar in both growth locations with glucose content decreasing over time (Fig. 5-4, C). In the 1983 experiments (Fig. 5-4, A and B) the pattern of glucose change was statistically different in the outer and inner scales. Synthesis and hydrolysis of sucrose as well as hexose isomerization might have contributed to the observed changes of glucose during storage. Moe and Wickstrom (1973) also found glucose to vary irregularly in tulip bulbs kept in storage. Rutherford and Weston (1968) working with Jerusalem artichoke tubers and with chicory and dandelion roots, observed no definite trends in the content of glucose of these organs with duration of cold storage. Working with Jerusalem artichoke tubers, Dorell and Chubey (1977) discussed the possibility of fructose being isomerized to glucose to provide the terminal glucose moiety for the synthesis of fructans.

Sucrose changes during storage were different in all the experiments. It increased up to 90 days in the Gainesville 1983 experiment (Fig. 5-6, A) probably partially due to the hydrolysis of oligosaccharides (Chapter IV). The observed decrease in sucrose content after 90 days might have been due to this sugar being utilized for the new growth. This trend was reversed in the Hastings 1983 experiment (Fig. 5-6, B). The general trend for sucrose during the second year experiments (Fig. 5-6,C) was to accumulate in the inner scales while decreasing in the outermost ones. This could have been due to the inward transport of this sugar. The decrease in sucrose levels in the inner and outer scales after 90 days might indicate mobilization of this sugar towards the growings roots. Rutherford and Weston (1968) found trends in sucrose levels to vary among different species during cold storage. In chicory the percentage sucrose increased throughout cold storage; in dandelion roots the sucrose content remained approximately constant; and in Jerusalem artichoke there was an initial rapid rise followed by a slight fall in the amount of sucrose later in storage.

The increase in dry weight from outer to inner scales (Chapter IV) was similar to that from previous studies (Darbyshire, 1978; Darbyshire and Henry, 1979; Mann and Hoyle, 1945). In spite of the differences in dry weight within the bulb(Chapter IV), mean values of the total nonstructural carbohydrate fractions of the outer and inner parts of the bulb showed only small differences when expressed as percentage of their corresponding dry weight (outer= 80%; inner= 74% dry weight). Darbyshire and Henry

(1979) found the ratio of nonstructural carbohydrate to percentage dry weight remained constant irrespective of final percentage dry weight.

In summary, the results from these experiments showed that sugars are unevenly distributed within the onion bulb, their concentration changing during storage. Factors such as bulb size, location within the bulb, growth location and cultivar had an effect on the observed results. The effect differed among sugars. The higher values of fructose in the outer scales might be indicative of more extensive hydrolysis of fructans in the outer bulb. This is in agreement with previous reports for onions grown in other geographical areas. Although fructans are known to yield fructose as the main product of hydrolysis (Edelman and Jefford, 1964, 1968), care should be taken in interpreting the observed changes in fructose during bulb storage in relation to fructan metabolism. Contribution of other sugars and physiological factors such as carbohydrate translocation and respiration add to the compexity of the overall carbohydrate balance of the stored bulb. Quantitative discrepancies between fructan and fructose changes were evident in the present experiments possibly due to the above mentioned reasons. The same situation seemed to occur for high latitude cultivars, although the quantitative differences were smaller.

In the present experiments, reducing sugars (fructose and glucose) constituted a larger fraction of the total nonstructural carbohydrates than that reported for cultivars grown in high latitudes. The opposite was found for sucrose. The different contribution of the individual sugars to the total nonstructural carbohydrate pool might be related to the observed differences in the storability of the cultivars grown at different latitudes. In the present experiment, lower levels of the individual sugars in the inner scales appeared to be related to better storability of the bulbs.

Fructose changes over time in the present experiments seemed to be a better indicator of the physiology of the stored bulbs when compared to the other sugars. Rutherford and Whittle (1982, 1984) found fructose levels at harvest and their changes during storage to be related to the storability of the bulbs. Sugar changes during storage might be used as biochemical indicators of the storability of the bulbs only if reliable and reproducible over seasons and locations.

CHAPTER VI
SUMMARY AND CONCLUSIONS

Fructans, fructose, glucose and sucrose constitute the major nonstructural carbohydrate reserves in onions where they are known to be related to the storability of the bulbs. These carbohydrates have been studied in more detail in high latitude cultivars which are known to be good keepers. The objective of this research was to study the metabolism of these sugars in relation to the storability of some low latitude onions. The levels and changes of the individual carbohydrates were monitored throughout development and storage of the bulbs.

The developmental study showed that fructans with degree of polymerization (DP) larger than 4 were present, in significant amounts, only in the bulb. Fructan levels in the leaf blades were markedly lower than in the bulbs. The predominant fructans found in leaf blades were those of DP3. Fructans with DP larger than 4 were not detected in leaves.

Carbohydrates accumulated significantly in the bulb, especially during the phase of fast development. The relative amounts of the individual sugars resembled that of the stored bulbs with the reducing sugars predominating.

The distribution of the different molecular weight fructans during bulb development was similar to that of the stored bulbs. Starch could not be detected in measurable amounts in the tissues analyzed which precludes it from being significant in the total nonstructural carbohydrate balance in quantitative terms.

The individual sugars were affected differently by factors such as cultivar, location within the plant or plant part, bulb size, growth location, and growth season. The sample size used in these experiments was large but the statistical analysis showed substantial variability. The largest differences in the values of the individual sugars and dry weight were detected in the lower part of the bulb.

Fructans changed during cold storage of the bulbs. The observed changes were different in the outer and inner scales with longer DP chains predominating and decreasing over time in the outer scales only. The relative distribution of the different DP fructans differed from that reported for onions grown in other geographical areas, but the general decreasing trend in the fructan levels of the outer scales was similar to that previously reported. Fructan levels in low latitude onions were markedly lower than those of high latitude cultivars. Storage trials carried out during 2 years with onions from the present experiments showed that higher fructan-containing bulbs appeared to have better storability.

Higher values of fructose in the outer scales seemed to indicate that fructans underwent hydrolysis to a larger extent in the outer bulb which is in agreement with the results found in higher latitude onions. Discrepancies in the levels of fructans and fructose over time evidenced the participation of other sugars in the overall balance of these carbohydrates. Other factors like carbohydrate translocation within the bulb and consumption through respiration probably contributed to this discrepancy.

Levels of reducing sugars were higher and those of sucrose and fructans were lower in low latitude onions as compared to those from cultivars grown in other geographical areas. Lower levels of fructose, glucose and sucrose appeared to be related to better storability of the bulbs.

The distribution of individual nonstructural carbohydrates within the bulb resembled that reported for high latitude cultivars. Fructans and sucrose increased centripetally whereas the opposite trend was found for fructose and glucose.

The data considered together suggest the following conclusions:

1) The levels of fructose, glucose, sucrose, and fructans in particular, seemed to constitute reliable indicators of the differences in storability in high and low latitude onions.

2) The molecular weight distribution and the levels of fructans differed in low latitude onions from that reported in higher latitude cultivars. Larger DP chains predominated and levels were markedly lower in the onions from these experiments.

3) Hydrolysis of fructans seemed to occur to a larger extent in the outer scales which agrees with the results from high latitude onions.

4) Fructose and glucose constituted a significantly higher percentage of the total nonstructural carbohydrate fraction of the bulb than corresponding values found in onions grown in other geographical areas.

5) Fructans accumulated in the bulb only and their molecular weight distribution was similar throughout its development and storage.

6) The differences in the levels of fructans found in the present experiments from those of high latitude onions together with the results of the storage trials indicated a possible role for these carbohydrates in the storability of the bulbs.

APPENDIX
SUMMARY ANALYSIS OF VARIANCE TABLES

Table A-1. Summary analysis of variance table for the stage of bulb development (date), plant part (pltpart), and location within the plant (horzt, vert) on the fructan, fructose, glucose, sucrose and dry weight content of 'Granex 429' onion plants grown at Hastings in 1984.

Source	Fructan	Fructose	Glucose	Sucrose	Dry Weight
Date	*[z]	***	***	***	***
Pltpart	***	***	***	***	***
Horzt	***	NS	***	NS	NS
Vert	***	***	**	***	***
PxD	*	**	**	***	NS
HxD	***	NS	NS	NS	NS
VxD	NS	NS	NS	NS	**
PxH	***	***	***	**	***
PxV	***	**	*	NS	***
HxV	**	**	*	**	***
PxHxD	NS	NS	NS	NS	NS
PxVxD	NS	NS	NS	NS	NS
HxVxD	***	NS	NS	NS	NS
PxHxV	***	**	**	NS	***
PxHxVxD	NS	NS	NS	NS	NS

[z] Significance levels: *, **, ***, and NS mean significant at P=0.05, P=0.01, P=0.001, and not significant, respectively.

Table A-2. Summary analysis of variance table for the effect of time of storage, location within the bulb and bulb size on the fructan, fructose, glucose, sucrose, and dry weight content of 'F_1 Yellow Granex PRR', 'Texas Grano 502 PRR', and 'Texas Grano 1030Y' onion bulbs grown at Gainesville in 1983.

Source	Fructan	Fructose	Glucose	Sucrose	Dry weight
Cultivar	NS^z	NS	NS	***	***
Bulb	***	***	**	***	***
Date	**	***	***	***	***
Size	***	NS	NS	NS	NS
CxB	NS	NS	NS	NS	NS
CxD	NS	NS	NS	NS	NS
CxS	NS	NS	NS	NS	NS
BxD	**	NS	*	NS	NS
BxS	*	**	*	NS	NS
DxS	NS	NS	NS	NS	NS
CxBxD	NS	NS	NS	NS	NS
CxBxS	NS	NS	NS	NS	NS
CxDxS	NS	NS	NS	NS	NS
BxDxS	NS	NS	NS	NS	NS
CxBxDxS	NS	NS	NS	NS	NS

^{z}Significance levels: *, **, ***, and NS mean significant at P=0.05, P=0.01, P=0.001, and not significant, respectively.

Table A-3. Summary analysis of variance table for the effect of time of storage (date) and location within the bulb (bulb) on the fructan, fructose, glucose, sucrose, and dry weight content of 'Granex 429'onion bulbs grown at Hastings in 1983.

Source	Fructan	Fructose	Glucose	Sucrose	Dry weight
Date	***[z]	***	*	**	**
Bulb	***	***	***	***	***
DxB	*	NS	**	NS	NS

[z] Significance levels: *, **, ***, and NS,mean significant at P=0.05, P=0.01, P= 0.001, and not significant, respectively.

Table A-4. Summary analysis of variance table for the effect of time of storage (date), location within the bulb (bulb), and growth location (location) on the fructan, fructose, glucose, sucrose, and dry weight content of 'Granex 429' and 'Texas Grano 1030Y' onion bulbs grown at Hastings and Gainesville in 1984.

Source	Fructan	Fructose	Glucose	Sucrose	Dry weight
Location	***[z]	***	NS	NS	***
Date	***	***	***	**	***
Cultivar	***	*	**	***	***
Bulb	***	***	***	***	***
LxD	NS	NS	**	NS	NS
LxC	NS	NS	NS	***	**
LxB	***	**	*	**	**
DxC	NS	NS	NS	NS	NS
DxB	*	NS	NS	***	**
CxB	**	*	NS	**	NS
LxDxC	NS	NS	NS	NS	NS
LxDxB	NS	NS	NS	NS	NS
LxCxB	NS	NS	NS	NS	**
DxCxB	NS	NS	NS	NS	NS
LxDxCxB	NS	NS	NS	NS	NS

[z] Significance levels: *, **, ***, and NS mean significant at P=0.05, P=0.01, P=0.001, and not significant, respectively.

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BIOGRAPHICAL SKETCH

Julio Toledo Hevia was born in Lima, Peru, on December 5, 1949. He graduated from the College of Agriculture, Universidad Nacional Agraria-La Molina, Lima, Peru, with a B. Sc. Agron. in 1973. Upon graduation, he joined the Department of Horticulture (Universidad Nacional Agraria) as a professor and researcher in the field of physiology and production of vegetable crops. He earned the degree of Master of Science in the Department of Vegetable Crops, University of California, Davis, in 1978. Mr. Toledo is married to Rita Rissi.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Richard C. Smith

R. C. Smith
Professor of Botany

I certity that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

JR Shumaker

J. R. Shumaker
Associate Professor of
Horticultural Science

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1985

Jack L. Fry

Dean, College of Agriculture

Dean, Graduate School

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Mark Sherman

M. Sherman, Chairman
Associate Professor of
Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

D. J. Huber
Associate Professor of
Horticultural Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

C. B. Hall

C. B. Hall
Professor of Horticultural
Science